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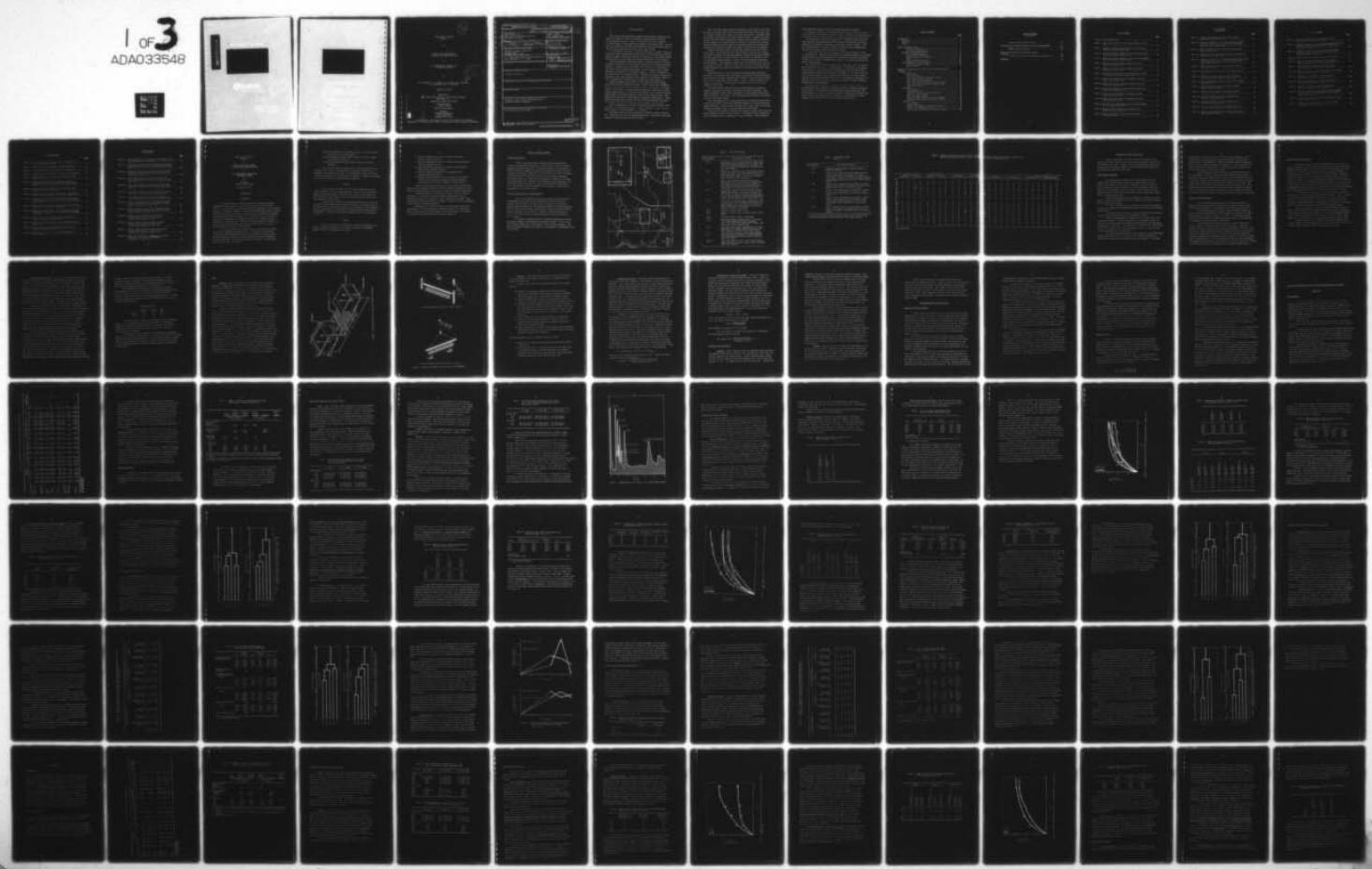
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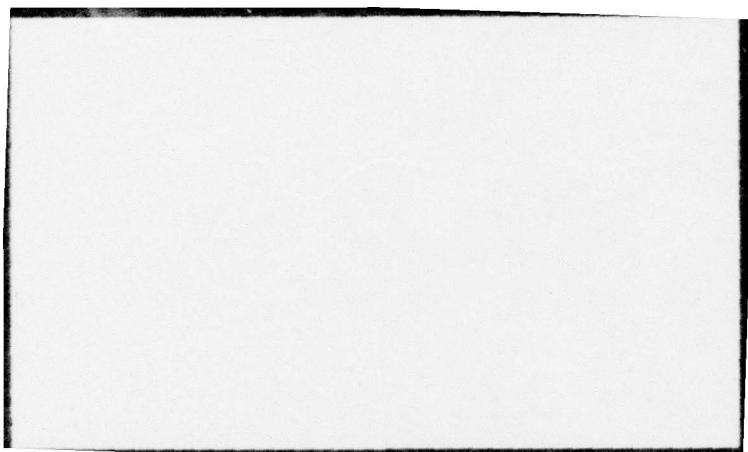
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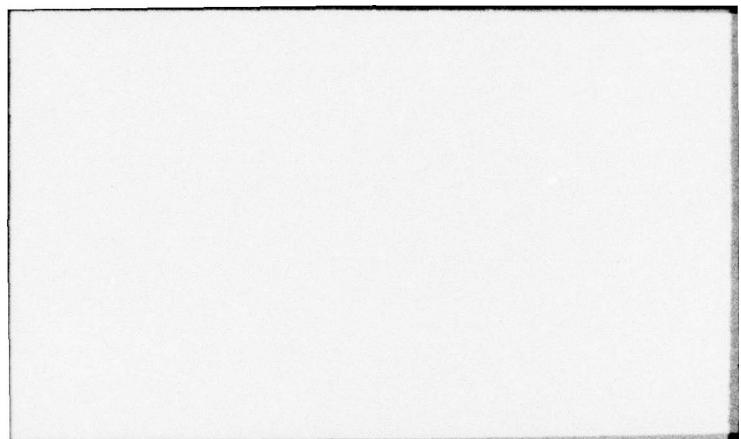
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**FINAL PHASE II REPORT  
(VOLUME I)**

on

AQUATIC LIFE FIELD STUDIES  
AT JOLIET ARMY AMMUNITION PLANT

to

**U.S. ARMY MEDICAL RESEARCH AND  
DEVELOPMENT COMMAND**

by

J. M. Stilwell, D. C. Cooper, M. A. Eischen, M. C. Matthews,  
B. E. Sherwood, and T. B. Stanford

December 10, 1976

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## EXECUTIVE SUMMARY

The United States Army Medical Research and Development Command has been supporting research in order to recommend environment quality standards for the munitions industry. Phase I preliminary studies were completed in 1974 and Phase II investigations were begun in the spring of 1975. Two surveys were conducted at the Joliet Army Ammunition Plant (JAAP) during Phase II, one in the late spring and the other in early fall of 1975.

The objectives of the Phase II studies at JAAP were to collect replicated quantitative data describing the specific nature of TNT manufacturing effluent effects on aquatic receiving systems and to determine the relationship between observed effects and the amount of munitions constituents in the effluents.

Areas investigated included water quality, sediment chemistry, munitions constituent concentrations, the periphyton community and the macroinvertebrate community. Periphyton and invertebrates were selected for study because of the relative ease of sampling and analysis of these groups as well as their usefulness as indicators of environmental quality. A variety of parameters were calculated for both groups studied. Standing crop, species diversity and colonization rates were obtained for both algae and invertebrates. Additionally, chlorophyll a and biomass were calculated from algae samples. A

Sampling stations were selected in three effluent receiving systems, Grant Creek, Prairie Creek and Doyle Lake. Control stations were sampled upstream of the outfalls in Grant and Prairie Creeks. No suitable control areas existed in Doyle Lake. Water and sediment samples were collected at all biological sampling stations. Periphyton was collected from artificial substrates (glass slides) and natural substrates (rocks) at all stream stations. Phytoplankton was collected from Doyle Lake with appropriate sampling gear. Benthic macroinvertebrates were collected from both artificial substrates (multiple-plate samplers) and natural substrates (Surber sample or Ponar dredge).

Field investigations at JAAP were conducted during April and June, 1975 and again in August-September, 1975. Intensive sampling occurred during the weeks of June 16 - 20 and September 15 - 19, 1975.

The primary munitions product discharged to aquatic systems at JAAP was TNT. Monitoring of TNT and two degradation products, 2,4-DNT and 2,6-DNT, was conducted at sampling sites and plant outfalls.

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Many of the water quality parameters measured in Grant Creek in the spring and fall surveys were higher than both regional levels and selected water quality criteria, primarily as a result of the two JAAP outfalls. Suspended solids are excessively high below the filtration plant outfall. Increased total hardness at this point persists downstream. Nutrient concentrations, particularly nitrogen species, are increased below the addition of the pink water effluent. Mean TNT concentrations were similar in the spring and fall and diminish downstream from the pink water outfall but appear to accumulate in the sediments.

Similar trends were evident in Doyle Lake (spring and fall) and Prairie Creek (fall). Both systems had high total dissolved solids and bore evidence of nutrient enrichment (nitrogen and phosphate species) from the LAP discharge. Mean TNT concentrations in water diminish from the point of discharge to low or undetectable levels downstream. However, TNT again appears to accumulate in the sediments. Other sediment characteristics in Prairie Creek are well below selected criteria. Sediments in Doyle Lake, however, have a high chemical oxygen demand and high nitrogen and phosphate concentrations which define them as "polluted".

The spring survey in Grant Creek provided excellent algae and invertebrate data. High flows at this time flushed the stream of the flocculant material contained in the water treatment plant effluent. During this survey little effect on the algae community was observed. However, noticeable changes were observed in the benthic macroinvertebrate communities below the pink water outfall to Grant Creek.

The fall survey was conducted during low flow conditions, allowing a buildup of flocculant precipitate over much of the bottom substrates. This created conditions in Grant Creek inhospitable to both periphyton and invertebrate colonization. The source of this perturbation was complicated by multiple outfalls to this small stream.

Doyle Lake sampling in the spring identified environmental stress existing in all areas studied. Only the phytoplankton community of the pond itself appeared unaffected. The fall survey reconfirmed this stress condition and demonstrated an enrichment effect on the phytoplankton of Doyle Lake.

Prairie Creek, surveyed in the fall only, showed the least effects from the plant discharge of all the systems studied. An enrichment effect was observed in the periphyton community downstream of the LAP effluent. Minor population

shifts were also observed in the benthic macroinvertebrate community.

Periphyton and benthic macroinvertebrates on natural and artificial substrates exposed to Pink Water Constituent ("PWC") concentrations ranging from zero to 10.0 ppb in Prairie Creek experience little or no adverse ecological effect.

Concentrations of "PWC" in the range of 50 - 100 ppb in Grant Creek and Doyle Lake water were associated with environmental perturbances reflected in periphyton, phytoplankton, and benthic invertebrate community changes. Sediment concentrations in the areas of greatest observed effects ranged between 10 and 40 times higher than those found in the water.

In conclusion, "PWC" (TNT, 2,4 DNT and 2,6 DNT) concentrations in water in the range of 50 - 100 ppb are associated with ecological changes in the benthic macroinvertebrate and algae communities of the aquatic ecosystems investigated. Therefore, a "no effect" threshold would appear to be at some concentration below 50 - 100 ppb range.

"No effect" levels of "PWC" probably lie in the range of 50 - 100 ppb for macroinvertebrates and algae. The transformation of "no effect" environmental concentrations to "no effect" effluent loading rates cannot be made without considering the effluent receiving system in question. In the case of TNT and its degradation products, their relatively high solubility in water (greater than the toxic levels indicated by this study) require specific knowledge as to dilution and dispersion in each receiving system before "no effect" effluent loading rates can be determined.

The relationship between "PWC" concentration and ecological response as measured by various changes in community structure or function is discussed in this report. These data and relationships were derived exclusively from field measurements, where a multitude of physical, chemical and ecological variables interact in an unknown manner. Therefore, no conclusions as to exact causality can be drawn from the results of this research effort.

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U.S. ARMY MEDICAL RESEARCH AND  
DEVELOPMENT COMMAND

from

BATTELLE  
Columbus Laboratories

February 15, 1976

INTRODUCTION

Background

The United States Army Medical Research and Development Command (USAMRDC) has been supporting a major research effort with the ultimate objective to develop suggested environmental quality standards for the munitions industry. Accordingly, USAMRDC is conducting and underwriting numerous research projects relating to the effects of numerous munitions compounds and effluents on aquatic and terrestrial organisms. These efforts include field studies to provide data supplementary to laboratory studies and to augment the interpretability of laboratory data. Battelle's Columbus Laboratories (BCL) is one of several contractors who have been engaged in field studies at various munitions plants in support of this objective.

BCL conducted Phase I screening studies at three munitions plants during 1974 to determine observable effects of munitions waste discharges on benthic macroinvertebrates and periphyton in effluent receiving systems, and to determine any indication of bioaccumulation of munitions compounds by aquatic organisms. The three facilities at which Phase I investigations were conducted were the following:

Badger Army Ammunition Plant (BAAP) (production of nitrocellulose, nitroglycerine, and rocket paste)

Joliet Army Ammunition Plant (JAAP) (production of TNT, loading and packing of Compound B)

Lake City Army Ammunition Plant (LCAAP) (production of primer compounds)

The results of Phase I investigations are described and discussed in Cooper et al. (1975). Observed effects of effluent discharges were suggested at BAAP, definite at JAAP, and indeterminate at LCAAP; there were no definite indications of bioaccumulation of munitions compounds by organisms in any of the effluent receiving systems investigated.

#### Purpose

Under Contract No. DAMD 17-74-C-4123 with USAMRDC, BCL has been conducting Phase II follow up investigations at two of the three munitions plants studied during Phase I - BAAP and JAAP. Phase II investigations at JAAP covered two survey periods, one in the spring of 1975, and one in the late summer-early fall of 1975.

The objectives of Phase II investigations have been to collect and analyze replicated quantitative information relative to the specific nature of effluent effects on receiving systems, and to determine the relationship(s) between observed effects and the amount of primary munitions constituents in the effluents. This report describes and discusses Phase II investigations at JAAP.

#### Scope

The scope of Phase II investigations at JAAP was predicated on the results of Phase I investigations and ensuing discussions with USAMRDC. Elements of Phase II investigations included:

- Replicate sampling and analysis of water and sediment from all sampling locations
- Replicate sampling and analysis of benthic macroinvertebrates from all sampling locations
- Replicate sampling and analysis of diatoms and filamentous algae from all sampling locations
- Replicate sampling and analysis of phytoplankton from selected sampling locations
- Analysis and interpretation of all resultant data relative to effluent effects and the association of primary munitions constituents (TNT, 2,4-DNT, 2,6-DNT) with these effects.

Benthic macroinvertebrate samples from both natural and artificial substrates were analyzed for number of species, number of individuals of each species, species diversity, and colonization rates of artificial substrates.

Periphyton samples were analyzed for number of species, number of individuals of each species, species diversity, colonization rates on artificial substrates, chlorophyll a, organic biomass and autotrophic index. Phytoplankton samples were analyzed for number of species, number of individuals of each species, and species diversity.

Discussion of the Phase II research strategy sample analysis and interpretation of results obtained is presented in Volume I of this report. Much of the raw data and calculated data, which provide support for Volume I is contained in the Appendices which constitute Volume II.

PHASE II RESEARCH STRATEGYFacility Description

JAAP is a government-owned contractor-operated installation located in northeastern Illinois about 15 miles south of Joliet, Illinois. Operated by Uniroyal Corporation, its primary mission is the manufacture of TNT and loading and packing of explosives including TNT and RDX. The plant facilities occupy about 20,000 acres of flat terrain, much of which consists of fallow old-fields or land under cultivation on a leasing arrangement with nearby farmers. Effluents from the manufacture of TNT flow into Grant Creek and Jackson Creek. Effluents from loading and packing operations flow into Prairie Creek and Doyle Lake, a small, man-made lake located onsite. All of these waters eventually discharge into the region of confluence of the Kankakee and Des Plaines Rivers.

Location and Description of Sampling Sites

Phase II JAAP sampling stations were located as shown in Figure 1. Corresponding station codes and site descriptions are found in Table 1. Station codes are based on acronyms of station location. "GC" prefixes indicate Grant Creek stations, "PC" prefixes indicate Prairie Creek stations, and "DL" prefixes indicate Doyle Lake stations. Numerical suffixes indicate location relative to other stations. Generally, control stations have a "-1" or "-2" suffix, whereas stations downstream of effluent outfalls are "-3" or greater.

The number of samples attempted, collected and analyzed of the various aquatic parameters investigated are presented in Table 2. Zeros in the table indicate samples were not attempted or were lost when attempted. Sediment and biological samples from outfalls were designated "not applicable" (N.A.) in this table.

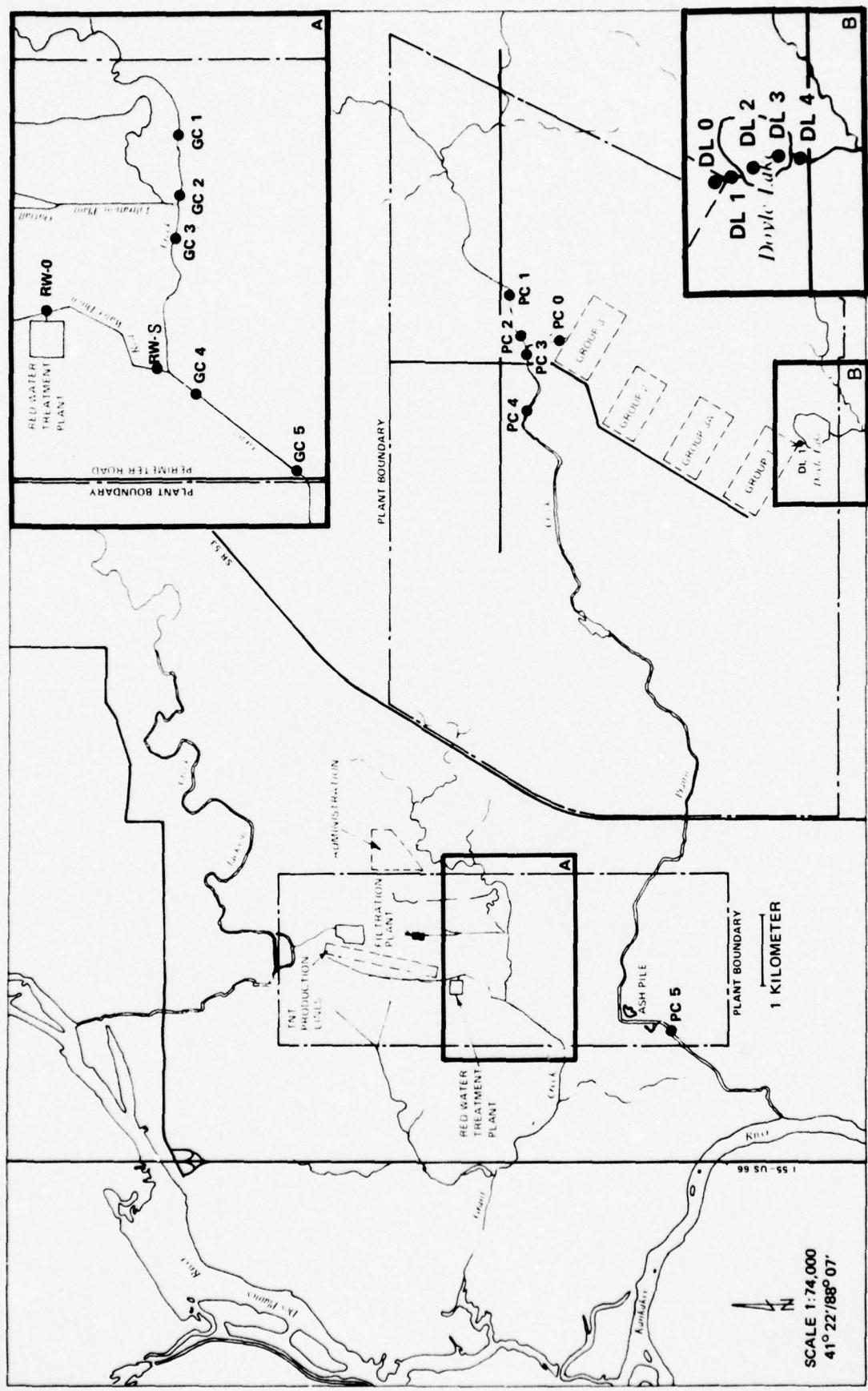


FIGURE 1. PHASE II SAMPLING STATIONS AT JOLIET ARMY AMMUNITION PLANT (JAAP), JOLIET ILLINOIS

TABLE 1. JAAP STATION LEGEND

| Station Number | Description of Station   |
|----------------|--|
| GC-1           | Grant Creek, upstream from filtration plant influx; intermittent; pools, separated by dry riffle areas during fall sampling period; pool - 3 m wide, 4 m long, 0.5 m deep, bottom substrate primarily sand, some gravel-pebble.  |
| GC-2           | Grant Creek, upstream from filtration plant influx; riffle - 3 m wide, 10 cm deep, sand-gravel bottom underlying cobble; pool - 5 m wide, 3 m long, 0.5 m deep, sand-gravel-cobble bottom; water in riffle areas was shallow and fast moving.  |
| GC-3           | Grant Creek, downstream from filtration plant influx; riffle - 1-2 m wide, 20-25 cm deep, sand-silt-pebble-cobble bottom with some detritus; water appeared milky due to white-gray flocculent suspended matter infiltration plant effluent.   |
| GC-4           | Grant Creek, downstream from pink water effluent; channalized; riffle - 3-4 m wide, 15-25 cm deep, pebble-cobble-rubble overlying sand bottom; pool - 5-6 m wide, 5 m long, 0.5 m deep, sand-silt-gravel bottom; exposed bedrock; algal growth observed on rocks; water was turbid, red to red-brown in color. |
| GC-5           | Grant Creek, at JAAP boundary; channalized; riffle - 3 m wide, 15-20 cm deep, pebble-cobble-rubble over gravel bottom; pool - 2-3 m wide, 2 m long, 0.5 m deep, organic muck to sand-gravel bottom; water appeared turbid and fast moving.   |
| RW-0           | Redwater treatment plant outfall.  |
| RW-S           | Redwater treatment plant discharge stream to Grant Creek.  |
| DL-0           | LAP line effluent outfall to Doyle Lake  |
| DL-1*          | Doyle Lake influent stream; riffle - 0.9 m wide, 1-5 cm deep, sand-gravel-cobble substrate; pool - 1.8 m wide, 2.5 m long, 25-30 cm deep, clay-sand bottom; water appeared clear.  |
| DL-2           | Doyle Lake settling pond; approximately 15 m from shoreline, 0.75 m deep; substrate clay-silt with large proportion detritus; water often turbid.  |
| DL-3           | Doyle Lake settling pond; located midway between inlet and outlet structures, 1 m deep; substrate fine silt mixed with clay particles and detritus; water very turbid.   |
| DL-4*          | Doyle Lake outlet; 3.0-4.0 m wide, 12-20 cm deep; muddy-silt layer covering a pebble-cobble substrate; water often turbid.   |

TABLE 1. JAAP STATION LEGEND  
(cont'd)

| Station Number | Description of Station  |
|----------------|---|
| PC-0           | LAP line effluent outfall to Prairie Creek, between PC-2 and PC-3.  |
| PC-1           | Prairie Creek, upstream from LAP Number 3 outfall; riffle - 1.0 m wide, 2-6 cm deep, 15 m long, gravel-pebble-cobble bottom; pool - 5 m wide, 0.5-1.0 m deep, 6 m long, silt-sand-gravel-pebble bottom; large <u>Lampsilis</u> naiads present; water clear; partial canopy. |
| PC-2           | Prairie Creek, upstream from LAP Number 3 outfall; riffle - 1-2.5 m wide, 2-8 cm deep, 3 m long, gravel-pebble bottom; water clear; partial canopy.   |
| PC-3           | Prairie Creek, downstream from LAP Number 3 outfall; riffle - 1.0-1.5 m wide, 4-8 cm deep, 6 m long, cobble-pebble-sand substrate; water clear; partial canopy.   |
| PC-4           | Prairie Creek, downstream from LAP Number 3 outfall; riffle - 4.0-5.0 m wide, 3-9 cm deep, 10 m long, gravel-pebble-cobble bottom; pool - 8-10 m wide, 0.75 m deep, 10-12 m long; water clear; partial canopy.  |
| PC-5           | Prairie Creek, downstream from LAP Number 3 outfall; riffle - 5 m wide, 8-10 cm deep; pool - 12-15 cm wide, cobble-pebble-sand-gravel; water clear; partial canopy.   |

\* Due to an alteration of plant effluent release, water flows at these stations were greatly reduced during the fall sampling period.

TABLE 2. NUMBERS OF SAMPLES OF DIFFERENT AQUATIC PARAMETERS  
ANALYZED FROM SAMPLE STATIONS AT JAAP, SPRING

|               | Chemical Characterization |          |                     |          | Munitions Constituents |          |           |          | Natural Substrates |          |           |      | Periphyton |           |
|---------------|---------------------------|----------|---------------------|----------|------------------------|----------|-----------|----------|--------------------|----------|-----------|------|------------|-----------|
|               | Water                     |          | Sediment            |          | Water                  |          | Sediment  |          | Attempted          |          | Collected |      | Attempted  | Collected |
|               | Collected                 | Analyzed | Collected           | Analyzed | Collected              | Analyzed | Collected | Analyzed | Collected          | Analyzed | N.A.      | N.A. | N.A.       | N.A.      |
| <b>SPRING</b> |                           |          |                     |          |                        |          |           |          |                    |          |           |      |            |           |
| GC-1          | 5                         | 3        | 3                   | 0        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| GC-2          | 5                         | 3        | 3                   | 0        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| GC-3          | 5                         | 5        | 3                   | 0        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| GC-4          | 5                         | 5        | 3                   | 3        | 5                      | 5        | 3         | 3        | 5                  | 5        | 5         | 5    | 9          | 2         |
| GC-5          | 5                         | 5        | 3                   | 3        | 5                      | 4        | 3         | 3        | 5                  | 5        | 5         | 5    | 9          | 9         |
| RW-0          | 5                         | 5        | N.A. <sup>(a)</sup> | N.A.     | 5                      | 2        | N.A.      | N.A.     | N.A.               | N.A.     | N.A.      | N.A. | N.A.       | N.A.      |
| RW-S          | 5                         | 5        | 0                   | 0        | 5                      | 5        | 0         | 0        | 0                  | 0        | 0         | 0    | 0          | 0         |
| DL-1          | 5                         | 5        | 3                   | 3        | 5                      | 3        | 3         | 2        | 5                  | 5        | 5         | 5    | 9          | 9         |
| DL-2          | 5                         | 3        | 3                   | 3        | 5                      | 3        | 3         | 2        | 5                  | 5        | 5         | 5    | 9          | 9         |
| DL-3          | 5                         | 3        | 3                   | 2        | 5                      | 3        | 3         | 2        | 5                  | 5        | 5         | 5    | 9          | 9         |
| DL-4          | 5                         | 3        | 3                   | 3        | 5                      | 4        | 3         | 2        | 5                  | 5        | 5         | 5    | 9          | 9         |
| DL-0          | 5                         | 5        | N.A.                | N.A.     | 5                      | 5        | N.A.      | N.A.     | N.A.               | N.A.     | N.A.      | N.A. | N.A.       | N.A.      |
| <b>FALL</b>   |                           |          |                     |          |                        |          |           |          |                    |          |           |      |            |           |
| GC-1          | 5                         | 2        | 3                   | 1        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| GC-2          | 5                         | 2        | 3                   | 1        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| GC-3          | 5                         | 4        | 3                   | 1        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| GC-4          | 5                         | 4        | 3                   | 1        | 5                      | 2        | 3         | 3        | 5                  | 5        | 5         | 5    | 9          | 9         |
| GC-5          | 5                         | 3        | 3                   | 1        | 5                      | 2        | 3         | 3        | 5                  | 5        | 5         | 5    | 9          | 9         |
| RW-0          | 4                         | 4        | N.A.                | N.A.     | 4                      | 4        | N.A.      | N.A.     | N.A.               | N.A.     | N.A.      | N.A. | N.A.       | N.A.      |
| DL-1          | 5                         | 2        | 3                   | 1        | 5                      | 2        | 3         | 1        | 5                  | 5        | 5         | 5    | 9          | 2         |
| DL-2          | 5                         | 2        | 3                   | 1        | 5                      | 2        | 3         | 1        | 5                  | 5        | 5         | 5    | 9          | 9         |
| DL-3          | 5                         | 2        | 3                   | 1        | 5                      | 2        | 3         | 1        | 5                  | 5        | 5         | 5    | 9          | 5         |
| DL-4          | 5                         | 2        | 3                   | 1        | 5                      | 2        | 3         | 1        | 5                  | 5        | 5         | 5    | 9          | 9         |
| DL-0          | 4                         | 3        | N.A.                | N.A.     | 4                      | 2        | N.A.      | N.A.     | N.A.               | N.A.     | N.A.      | N.A. | N.A.       | N.A.      |
| PC-1          | 5                         | 2        | 3                   | 0        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| PC-2          | 5                         | 2        | 3                   | 1        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 5    | 9          | 9         |
| PC-3          | 5                         | 3        | 3                   | 1        | 5                      | 2        | 3         | 3        | 5                  | 5        | 5         | 5    | 9          | 9         |
| PC-4          | 5                         | 2        | 3                   | 1        | 5                      | 2        | 3         | 3        | 5                  | 5        | 5         | 5    | 9          | 9         |
| PC-5          | 5                         | 0        | 3                   | 1        | 5                      | 0        | 3         | 0        | 5                  | 5        | 5         | 1    | 9          | 9         |
| PC-0          | 4                         | 3        | N.A.                | N.A.     | 4                      | 2        | N.A.      | N.A.     | N.A.               | N.A.     | N.A.      | N.A. | N.A.       | N.A.      |

(a) N.A. - Not Applicable

(b) Phytoplankton Sample

T AQUATIC PARAMETERS ATTEMPTED, COLLECTED AND  
AT JAAP, SPRING AND FALL 1975.

Sampling and Analytical Methods

Intensive sampling of the aquatic systems receiving munitions wastes in the area of the JAAP was conducted during the weeks of June 16-20, and September 15-19, 1975. Samples of water and sediment were collected in conjunction with biological sampling of the benthic macroinvertebrate and algae communities inhabiting these areas.

Water Sample Methodology

Each water sample collected at JAAP was analyzed on-site for conductivity, dissolved oxygen, temperature, and pH by means of appropriate portable probes and electrodes. Laboratory analyses of selected samples returned to Battelle include total solids, dissolved solids, suspended solids, alkalinity, hardness, chloride, sulfate, nitrate, nitrite, ammonia, Kjeldahl nitrogen, total phosphorus, chemical oxygen demand (COD), and total organic carbon (TOC).

Chloride concentrations were determined by colorimetric titration using an Aminco-Cotlove Chloride Titrator. Ammonia was determined using an Orion ion-selective electrode.

All forms of phosphorus in each sample were converted to phosphate and the total phosphorus then determined according to the recent EPA published methods (1973).

Sulfate analyses were made by titration using the barium perchlorate procedure originally developed by Fritz and Freeland (1954).

Chemical oxygen demand was determined in accord with the EPA published methods (1973). For the determination of total organic carbon, an aliquot of each water sample was acidified with hydrochloric acid and purged with nitrogen to remove CO<sub>2</sub>. The sample was then injected into a Beckman TOC Analyzer and combusted under oxygen atmosphere at 900 C to yield CO<sub>2</sub>, which was then quantified with an IR detector.

All remaining analyses were conducted according to the APHS standard methods described by Taras et al. (1971). The values for hardness and alkalinity are expressed as equivalents of calcium carbonate. The hardness

determination is actually a measure of both calcium and magnesium, that is, total hardness, and the alkalinity values are based on a titration with sulfuric acid. A few samples were acidic upon collection and the alkalinity value in those instances represent the equivalents of calcium carbonate that would be required for neutralization. These samples are designated in the water quality tables in the Results section.

Time differentials between collection and analysis varied due to the large number of samples to be analyzed and staged collection periods. During the interim, all samples were kept under constant refrigeration (4 C) in the dark. Further, three separate sample bottles were used for collection at each station and chemical preservatives were added to two of them. The sample collected for nitrogen and phosphorus analyses was placed in a 200 ml polyethylene bottle, preserved with 10 mg of mercuric chloride, and refrigerated. The third water sample collected for all other analyses was placed in a 500 ml polyethylene bottle and refrigerated with no chemical preservatives.

#### Sediment Sample Methodology

Selected sediment samples collected at JAAP were submitted to Battelle for laboratory analysis of total solids, volatile solids, nitrate, nitrite, Kjeldahl nitrogen, total phosphorus, and chemical oxygen demand.

The phosphorus analysis involved fusing the sediment sample with  $\text{Na}_2\text{CO}_3$  followed by quantification of total phosphorus according to the APHS standard methods described by Taras et al. (1971). All remaining analyses were performed according to the APHS standard methods also, except COD which was analyzed by the published EPA method (1973).

Time differentials between collection and analysis varied due to the large number of samples and staged collection periods. During the interim, all samples were kept under constant refrigeration (4 C) in the dark. Further, two sample bottles were used for sediment collection at each station with chemical preservation of one. The sample to be used for analysis of nitrogen, phosphorus and COD parameters was collected in a 200 ml polyethylene bottle, preserved with 0.5 ml of concentrated sulfuric acid, and refrigerated. The sediments to be analyzed for total solids and volatile solids were collected in 250 ml glass bottles and refrigerated with no chemical preservatives.

Munitions Constituent Analysis

This study included the accurate determination of low levels of munitions constituents in the production effluents, stream water and sediment samples collected at JAAP in conjunction with biological sampling and assay. The munitions selected for analysis were 2,6-dinitrotoluene (2,6-DNT), 2,4-dinitrotoluene (2,4-DNT), and 2,4,6-trinitrotoluene (TNT). These were selected for study as the most important waste materials generated in the production of TNT. 1,3,5-trinitrohexahydro-sym-triazine (Cyclonite, RDX) was also proposed for study. However, satisfactory methodology was not developed to permit quantitation of low levels of this munition in a simultaneous gas chromatographic analysis with TNT. Cost limitations did not permit development and multiple sample analysis for RDX in a separate analytical procedure.

The munitions compounds selected for study show some sensitivity to photolytic as well as chemical and thermal degradation. Hence, all samples were collected in amber bottles, shipped in wet ice filled coolers, and stored at 4 C prior to analysis. The sample sizes collected were 500 ml for water and approximately 250 ml (300-400 gms) for sediment. No preservatives or inhibitors were added to these water and sediment samples.

Solvents used in this study were distilled-in-glass analytical grade obtained from Burdick and Jackson Laboratories, Muskegon, Michigan. Chromatographic analyses were performed on a Varian 1700 Aerograph equipped with a Sc<sup>3</sup>H electron capture detector equipped with an Infotronics Model CRS-204 digital integrator and a Hewlett-Packard Automatic Sampler 7670A.

Standard solutions of the munitions studied were prepared from authentic samples obtained during Phase I and II of this study from Dr. B. E. Hackley, Edgewood Arsenal, Aberdeen Proving Ground, Maryland.

2,6-DNT, 2,4-DNT, and TNT. The mono, di, and trisubstituted nitrotoluenes are in general crystalline materials with low solubility in water. Solubilities of 270 ppm, 270 ppm and 130 ppm of 2,6-DNT (mp. 66 C), 2,4-DNT (mp. 70-1 C), and TNT (mp. 82 C) respectively, are reported near 20 C. The method of analysis of the munitions utilized in this study was suitable for detecting concentrations down to 0.05 ppb for the two DNT isomers, and 0.2 ppb for TNT. However, the inability to accurately reproduce a measurement of peak area prevented accurate determination of low concentrations. Based on the worst instance, measurement of concentrations in water sampled were not reported below 0.4 ppb for 2,6-DNT, 0.2 ppb for 2,4-DNT, and 0.6 ppb for TNT. Limits of five times these values were established for sediment samples.

The procedures used in the analysis of TNT and the DNT isomers in water samples were patterned after those described by Crook (1972) and Hoffsommer and Rosen (1972), and the Phase I procedure. Water sample aliquots of 100 ml each were extracted with two 40 ml portions of benzene. After the addition of an internal standard (1,5 dinitronaphthalene), the extracts were reduced in volume to 2.0 ml by rotary evaporation in the dark at 45 C. Sediment samples were extracted with 250 ml of benzene 12-15 hours in a Soxhlet extractor in the dark, spiked with internal standard, and then reduced in volume to 2.0 ml. The concentrated extracts of both water and sediment samples were then analyzed via electron capture gas chromatography.

The procedure used for analyzing the waste stream water and sediment samples permitted accurate quantitation within the range of a few parts per billion. Concentrations in water samples were determined from two separate extractions of each water sample. However, sediment sample analysis was normally based on only a single such extraction. The photolytic sensitivity of these munitions increases significantly above room temperature. Hence, all rotary evaporations were performed in the absence of light and the extracts were stored in a refrigerator. Chromatographic analysis of a 2-ml benzene solution of ~10 ppm each of 2,4 and 2,6 DNT, and TNT which had been exposed to sunlight for 24 hours, revealed the complete absence of these munitions and the formation of unknown material(s) with a retention time of about

8 minutes. However, this unknown species did not appear to be a major constituent in any of the water or sediment samples examined. A more extensive study would be required to determine the exact nature and fate of the photolytic decomposition product(s) of these munitions.

The reliability of this method was determined by subjecting known amounts of TNT, and 2,4- and 2,6-DNT to the analytical procedure used for water samples. The results, given below, indicate 100 percent recovery of these munitions in the 1-10 ppm concentration range. A recovery efficiency study was not possible with sediment samples due to the lack of a "clean" sediment matter.

Percent Recovered

|        | <u>2,6-DNT</u> | <u>2,4-DNT</u> | <u>TNT</u> |
|--------|----------------|----------------|------------|
| 10 ppm | 98             | 105            | 100        |
| 1 ppm  | 103            | 98             | 94         |

Gas chromatographic analysis of these munitions was accomplished using a 6 ft x 2 mm glass column packed with 5% SE-30 on Chromosorb W, maintained at a column temperature of 160 C (isothermal). The injector temperature was 200 C, the detector temperature was 220 C and the N<sub>2</sub> carrier gas flow = 30 ml/min.

Quantitation of these munitions was accomplished by determining the ratio of peak areas of the munitions to an internal standard in each sample. Prepared mixtures of measured amounts of the munitions and internal standard were run in conjunction with a series of samples, and the peak area ratios of these standard mixtures were used to determine munition concentrations in the samples.

Algae

Sampling. Natural substrates from all JAAP stream stations were sampled for attached algae (periphyton) during both main survey periods - June 16-20, and September 15-19, 1975. Five replicates were collected at each station. Scrapings of a known area ( $3.14 \text{ cm}^2$ ) were made on cobble-sized rocks with a knife, funneled into a sample bottle and preserved with 6:3:1 - an algal preservative of 60 percent water, 30 percent alcohol, 10 percent formalin. Five replicate samples of phytoplankton were also collected from the near shore and middle pond stations in Doyle Lake. Subsurface samples were taken in 2 liter volumes, filtered through a Wildco 20-mesh plankton bucket, rinsed into bottles and preserved with 6:3:1.

Artificial substrate periphyton samplers (diatometers) were set out at each Grant Creek and Doyle Lake sampling station on June 3, 1975, for the spring survey, and on September 2, 1975, for the fall survey. Samplers were placed in Prairie Creek on September 3, 1975. Each sampler consisted of a vertically oriented 15-unit slide rack maintained at constant water depth of 10 cm by floats which also served as wing guards to prevent fouling and scouring (Figure 2). The samplers were attached by nylon rope to the float on the invertebrate artificial substrate sampler unit (Figure 3). Samplers were incubated in situ for a period of 3 weeks. Slides were removed from the diatometers at 1-week intervals; 2 slides the first week, 5 the second, 2 the third. The collection of the 5 slides occurred during the main survey period. All slides for periphyton analysis were placed in individual sample bottles and preserved with 6:3:1. Six slides were also collected during the main survey period for chlorophyll a and ash-free dry weight biomass analyses. These slides were placed in a single sample bottle, immediately wrapped in foil and packed in ice to prevent further photosynthetic activity and to minimize chlorophyll breakdown. Samples were then placed in a freezer within 4 hours of collection and remained frozen until analyses were performed.

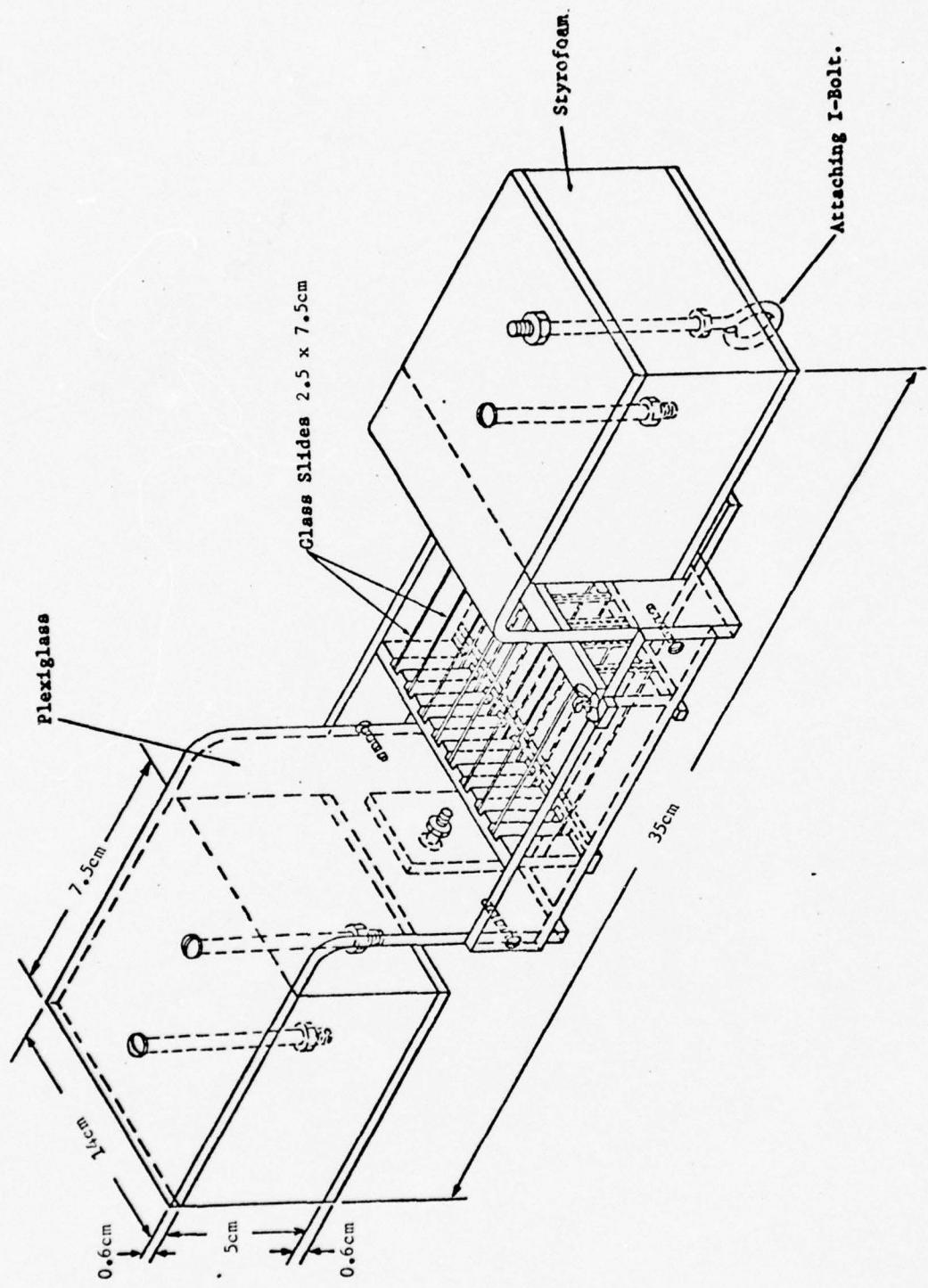
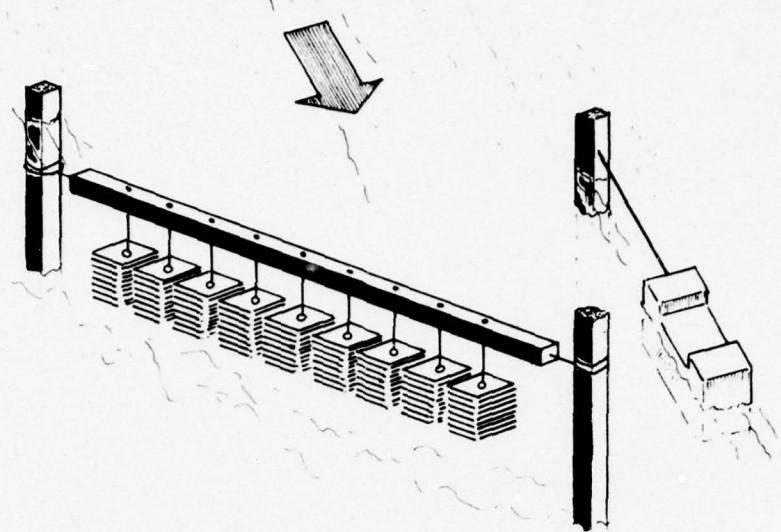
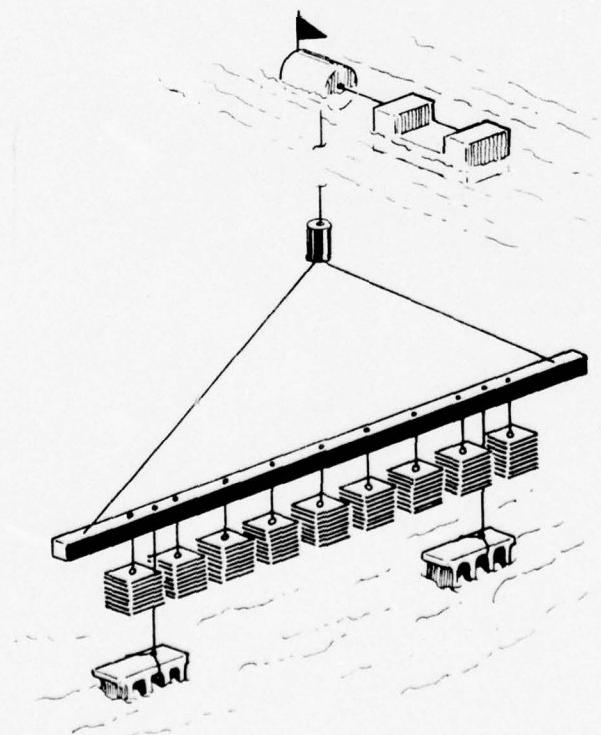


FIGURE 2. ARTIFICIAL SUBSTRATE PERIPHYTON SAMPLER (DIATOMETER)



A. Incubation Rack Orientation at Lotic Stations



B. Incubation Rack Orientation at Lentic Stations

FIGURE 3. ARTIFICIAL SUBSTRATE INCUBATION RACK AND DIATOMETER

Analysis. Quantitative procedures were utilized in the analysis of all algae samples. All samples were adjusted to a constant volume to normalize resultant data.

Diatoms in the samples were analyzed according to the following procedure:

- Fifteen ml of sample were removed with a pipette from each sample bottle and treated with 30% H<sub>2</sub>O<sub>2</sub> and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> to dissolve extraneous organic matter (including non-diatomaceous algae).
- Residue was washed with tap water; the supernatant liquid was decanted 3 to 5 times at 3-hour intervals until the sample cleared.
- Ten drops (0.5 ml) of sample were placed on a cover slip; water was evaporated from the cover slips on a slide warmer; cover slips were then placed on a hot plate for a minimum of 2 hours to combust any organic material which may have remained in the diatom frustules.
- Cover slips were fixed to slides using Hyrax mounting medium
- Diatoms on each slide were identified to species under oil immersion with the aid of appropriate taxonomic keys (Hustedt, 1930; Patrick and Reimer, 1966).
- Counts were made and recorded for 40 microscope fields (averaging approximately 200 cells; in the case of sparsely populated samples, the entire slide was often counted).

For non-diatomaceous algae, a separate procedure was used:

- One-tenth ml of sample was transferred by pipette into a Palmer counting cell.
- Whole-organism counts of 40 Whipple-grid fields were made using a compound microscope at 400X and appropriate keys enabling identification to the lowest practical taxon of non-diatom forms (Prescott, 1962; Smith, 1950; Taft and Taft, 1971).

Chlorophyll a Analysis. Chlorophyll extraction procedures following Weber (1973) were conducted in near darkness to prevent further photosynthesis. Only a small indirect light was used. Regent grade acetone was diluted by filling a 100-ml graduated cylinder with 90-ml of acetone and adding sufficient distilled water to bring the resultant mixture to 100-ml. Surgical gloves were used to hold the thawed slides while the organic accumulation from each of the six slides from one site were scraped into the same evaporating dish. The slides and field sample bottle were rinsed with 88 percent acetone; the scrapings were poured into a 60-ml glass vial. The evaporating dish was also rinsed with 88 percent acetone and poured into the glass vial, which was topped off with 88 percent acetone. The algal cells were disrupted for 5 minutes using a Sonifier Cell Disrupter (Model W185 by Heat Systems - Ultrasonics, Inc.) and allowed to steep for 24 hours at 4°C to facilitate further phytopigment extraction.

Chlorophyll a analysis was made of the combined extracts from the scrapings of the six replicate slides. Depending on the clarity of the sample, 10, 20 or 30 ml of extract were transferred to a centrifuge tube; and 88 percent acetone was added to the centrifuge tube (more acetone was used for darker samples) to make a total of 3 ml. Samples were centrifuged at 1600 rpm's at 4°C for 15 minutes. The optical density of 3 ml of supernatant was measured at 663, 645, and 630 nanometers (nm) in a Model B Spectrophotometer (Beckman Instruments, Inc.), using a 88 percent acetone blank for standardization. The contents of the centrifuge tube were thoroughly mixed and returned to the original sample bottle. The centrifuge tube was rinsed with 88 percent acetone, and this was also added to the sample bottle. Corrections for turbidity were made by determining OD 750 and subtracting these values from OD 663, OD 645, and OD 630. Chlorophyll a concentrations were calculated using the following formulas:

$$C_a = 11.64 \text{ OD}_{663} - 2.16 \text{ OD}_{645} + 0.10 \text{ OD}_{630},$$

where  $C_a$  is the chlorophyll a concentration in mg/l. This was converted to area of substrate ( $\text{mg Chl}_a/\text{m}^2$ ) by:

$$\text{mg Chl}_a/\text{m}^2 = \frac{C_a (\text{mg/l}) \times \text{volume of extract (l)}}{\text{area of substrate (\text{m}^2)}}$$

Determination of Ash-Free Dry Weight. Ash-free dry weight was determined following the methods described by Weber (1973). Contents of the 60-ml vial and centrifuge tube used in chlorophyll a analysis of each sample were poured into an evaporating dish, which had been previously washed with concentrated hydrochloric acid, burned in a muffle furnace at 550 C, allowed to cool in a dessicator for 1 hour, and weighed on an analytical balance. The dish with the combined extracts from the six replicate scrapings was dried in an oven at 105 C for a minimum of 24 hours, placed in a dessicator to cool for 1 hour, and then weighed on an analytical balance. The dried samples were ashed in a muffle furnace at 550 C for 1 hour, allowed to cool, rewetted with approximately 2 ml of distilled water to replace water of hydration lost when the sample was ashed, dried again in a drying oven at 105 C for 24 hours, cooled in a dessicator for 1 hour, and finally weighed on an analytical balance. Ash-free dry weight was determined using the following formula:

$$\text{g/sample} = \text{g dry} - \text{g ash wt}$$

This is converted from  $\text{g/mm}^2$  to  $\text{mg/m}^2$  (the sample weight was divided by six to give the weight of one slide) by the formula:

$$\text{mg/m}^2 = \frac{\text{g/sample} \times 1000}{(6)(.003871)}$$

where 0.003871 is the area in  $\text{m}^2$  of one slide.

Once the biomass of the algae was determined, the autotrophic index was computed using the formula:

$$\text{autotrophic index} = \frac{\text{ash-free wt } (\text{mg/m}^2)}{\text{chlorophyll a } (\text{mg/m}^2)}$$

#### Benthic Macroinvertebrates

Sampling. Benthic macroinvertebrates inhabiting natural substrates were sampled at JAAP during June 18-19, 1975, and September 17-19, 1975, with a Surber sampler at all stream stations. Samples from Doyle Lake stations DL-2 and DL-3 were collected from bottom sediments with a Petite Ponar grab sampler (6" x 6"). Five replicates were taken at each station. Samples were

immediately screened to remove silt and debris, placed in a plastic bottle stained with Rose Bengal, and preserved 24 hours later in 60 percent ethanol.

Multiple-plate artificial substrate samplers (Hester and Dendy, 1962) were employed at all sampling stations to collect benthic macroinvertebrates. Nine samplers were suspended from incubation racks just above the bottom substrates (Figure 3, A and B). The incubation racks were set in place at each sampling station during the spring sampling period on May 19, 1975. These substrate samplers were then retrieved at 3-, 4-, and 5-week incubation periods. Artificial substrates for the fall sampling period were set out August 26, 1975, and retrieved at 2-, 3-, and 4-week incubation intervals. Two samplers were removed after the first incubation period, five samplers were retrieved during the main survey period, and the remaining two were collected one week later.

Two techniques were used at JAAP to suspend and collect the artificial substrate samplers. At lotic (stream) stations, samplers were set in place by attaching the incubation rack to stakes which were driven into the bottom substrates (Figure 3, A). The appropriate samplers were cut from the rack and immediately placed in 1-quart plastic containers. In the lentic habitat at Doyle Lake, the incubation rack was suspended by floats and anchored in position with cinder blocks (Figure 3, B). The samplers were collected by carefully raising the entire incubation rack to within a few centimeters of the surface, cutting the appropriate samplers from the rack, and slowly returning the rack to position. Samplers were placed in plastic containers and sealed. All samplers were completely disassembled and thoroughly washed into a 30-mesh screen at a JAAP facility. Organisms removed from each sampler were placed in individual bottles, preserved in a 5% formalin solution, and returned to BCL for identification and enumeration.

Analysis. Organisms in unsorted samples were hand-picked in the laboratory from the debris. Bottom-grab samples from Doyle Lake stations DL-2 and DL-3 were washed in the laboratory to remove the remaining silt and excess Rose Bengal from the samples. Artificial substrate samples were also washed to remove the preservative. All samples were placed in glass petri dishes for identification and enumeration.

Benthic macroinvertebrates were identified with the aid of stereoscopic and compound microscopes. Identification to the lowest practical taxon was made utilizing taxonomic keys (Eddy and Hodson, 1961; Edmonson, 1959; Heard and Burch, 1966; Mason, 1968; Pennak, 1953; Peterson, 1960; Usinger, 1971; Walter and Burch, 1957; Johannsen, 1933; Ross, 1944; Frison, 1935; Burks, 1953). Midge larvae were mounted on slides and identified under a compound microscope (400X) in accordance with procedures presented by Mason (1968).

#### Data Management and Interpretation

##### Analysis of Variance (ANOVA)

One-way analyses of variance were performed on benthic and algae sample data to test for significant locational differences in the following three measures of community structure: (1) population density or standing crop (expressed as numbers of organisms per sampling unit), (2) numbers of species per sampling unit, and (3) Shannon diversity index ( $\bar{H}$ ). Artificial and natural substrate data were always analyzed separately; further, the data from the artificial substrates were analyzed separately for each of the three different sampling weeks and with respect to sampling season (spring or fall).

Data from Grant Creek and Prairie Creek were used in separate analyses of variance to test for differences between sites located upstream and sites located downstream of munitions outfalls. At Grant Creek, each of the five sampling stations was treated as an individual group in the analyses of variance, which tested all five stations simultaneously for differences among themselves.

At Prairie Creek, data from the upstream stations PC-1 and PC-2 were pooled together to form one group mean, as these two stations were located close to one another; similarly, the downstream stations PC-3 and PC-4 were pooled to form a second group mean. This pooling was done to increase the power of the ANOVA to detect significant upstream - downstream differences, because obvious differences were not anticipated. Data from PC-5 was not used

in any analyses of variance, due to its distance downstream from the Group 3 outfall and its different morphometry.

The analyses of variance were performed on Battelle's CDC 6400 computer system, using the SPSS Statistical programs (Nie, et al., 1975). An F-test having a significance level of 0.90 or greater was chosen as the criterion for deciding whether the group means were different. The simplest method for presenting the results of the ANOVA is to display the significance level of the F-test, and the group means accompanied by the line segments generated from a Duncan's Mean Separation Test.

The Duncan's Mean Separation Test (Duncan, 1955) is a necessary follow-up to an ANOVA whenever three or more means are demonstrated by the ANOVA to be significantly different, because it determines which particular group means are significantly higher or lower than which others. The Mean Separation Test arranges the means in increasing order from top to bottom. Line segments which illustrate the similarities and differences among them are drawn vertically to the immediate right of the means. If any two means are connected by a line segment, they are similar. If none of the line segments connects both of them, the two means are shown to be significantly different, with the higher of the two means being significantly higher than the lower one.

The Mean Separation Test is relevant only if the preceding ANOVA testing for differences among means is significant at a level of 0.90 or greater. If the ANOVA is not significant, the Mean Separation Test is redundant because it merely connects all the group means together with one line segment, restating that they are similar. Also, the Mean Separation Test provides no new information when there are only two group means being tested in the ANOVA, as in the case of Prairie Creek--the upstream group versus the downstream group. If the ANOVA shows significant differences between the two groups, it automatically follows that the higher of the two means is significantly higher than the lower of the two.

It must be noted in general that in order for group means to be proven significantly different by an ANOVA, the values within each group must be consistent enough so that within group variability is reasonably small. Large within-group variability may produce differences among means that are only the result of a few extreme fluctuations within some particular groups, rather than consistently occurring values. This non-uniformity, or heterogeneity, of group variances may cause an F-ratio to be not significant even though the magnitude of the differences among the group mean may appear to be quite large.

To check for such an occurrence, the hypothesis of homogeneity of group variances was tested using the F-max statistic to see whether the data fulfilled this assumption required by the ANOVA. The F-max statistic is the ratio of the largest to the smallest within-group variance. For numbers of species and diversity indices, this hypothesis was accepted, but for raw numbers of individuals it was often rejected. The numbers of individuals were transformed by logarithms and retested; the transformation succeeded producing homogeneous variances, so these log data were used in the ANOVA's. It must be noted, however, that the means of raw numbers are presented in the tabled results so that they may be more easily interpretable.

#### Cluster Analysis

In order to measure the degree of similarity between sampling sites with respect to species assemblages, similarity coefficients were computed among all possible pairs of sites for benthos and algae data. The term "species assemblage" will be used to jointly refer to two characteristics of a biological population--species composition, and the abundance of organisms within each of those species present.

Separate similarity coefficient matrices were computed for artificial and natural substrate data, and for spring and fall data. The similarity coefficient used was the Pinkham and Pearson measure of evenness (Pinkham and Pearson, 1974), and is defined as

$$S_{ab} = \frac{1}{k} \sum_{i=1}^k \frac{\min(X_{ia}, X_{ib})}{\max(X_{ia}, X_{ib})}$$

In this expression  $S_{ab}$  = similarity between sites a and b, k = the number of species found at one or both sites,  $X_{ia}$  = the number of individuals of species i at site a, and  $X_{ib}$  = the number of individuals of species i at site b. The values used for the X's were obtained by pooling together the individuals of species i over all the replicates within each sampling site for artificial substrates; all replicates over all three sampling weeks were pooled. Mutual absences of species, that is, values of i where both  $X_{ia}$  and  $X_{ib}$  were zero, were ignored, since the long species lists involved could lead a pair of sampling sites with no species in common to have a high similarity coefficient simply by having a large number of mutually absent species.

The value of  $S_{ab}$  can range from a minimum of 0 to a maximum of 1.  $S_{ab} = 0$  where sites a and b have no species in common;  $S_{ab} = 1$  when a and b have exactly the same species present and each of these species has the same density at site a as it does at site b. This is such a stringent requirement that it is very rarely achieved in actual field data. As a consequence, all the  $S_{ab}$  values calculated were rather low even for closely located pairs of sites. However, much information can be gained from studying the relative sizes of these coefficients.

The calculated similarity matrices were used in a computer program (Anderberg, 1973) which performed cluster analyses to link the sampling sites into a hierarchical "tree" structure, which is displayed in the form of a dendrogram. The clustering algorithm used was the unweighted pair-group method which maximizes the average similarity index between the merged pairs of groups (Kaesler and Cairns, 1972).

Similarity coefficients provide a way of comparing and contrasting sampling sites which is different from that provided by analyses of variance. Two sites could be demonstrated by an ANOVA and a Mean Separation Test to be similar on the bases of numbers of individuals, numbers of species, and species diversity; yet, it is still conceivable that these two sites could have completely different species compositions from one another. On the other hand, a similarity coefficient directly measures the degree of overlap in species composition and species dominance between two sites because its computation utilizes information about the simultaneous occurrences of various species.

RESULTS OF INVESTIGATIONS IN "PINK WATER" EFFLUENT RECEIVING SYSTEMSGrant CreekWater Quality

Table 3 is a summary of the water chemistry analysis from the spring and fall sampling effort in Grant Creek. The complete data sets utilized to compose this summary table appear in Appendix A, Tables 1 and 2. The sample sites are described in an annotated legend (see Table 1) and located on Figure 1. The table also includes selected water quality criteria (Battelle Columbus Laboratories, 1975a and 1975b; Eckenfelder, 1970; National Science Foundation, 1973) which serve as references in the following discussion of water quality.

Many of the water quality parameters investigated in Grant Creek were found to be both regionally high and in excess of selected water quality criteria.

As represented by a mean of 476 mg/l, dissolved solids concentrations at the Grant Creek control stations (GC-1 and GC-2) were slightly higher than expected for drainage systems near the Des Plaines and Kankakee Rivers confluence (Battelle Columbus Laboratories, 1972; U.S. Environmental Protection Agency, 1974). In comparison to the selected criteria, suspended solids and nutrients are also high at GC-1 and GC-2.

Data from GC-3 reflects the influence of the industrial water treatment plant discharges. Water treatment condensates discharged to the creek were responsible for considerable increases in sulfate, chloride, nitrite/nitrate concentrations during both sampling periods. During low flow conditions of the fall survey, mean dissolved solids increased to 1,780 mg/l at GC-3 - more than three times the control conditions and desirable regional water quality criteria. Total hardness remained over 450 mg/l downstream at GC-4 and GC-5 during the fall.

TABLE 3. WATER QUALITY DATA FROM GRANT CREEK, SPRING AND FALL, 1975

|                                       | Field Measurements |       |             |       |      |             | Laboratory Measurements (ppm) |                 |         |         |         |                 | Number of Samples Considered<br>Lab Field |                 |                 |                 |        |        |    |
|---------------------------------------|--------------------|-------|-------------|-------|------|-------------|-------------------------------|-----------------|---------|---------|---------|-----------------|---|-----------------|-----------------|-----------------|--------|--------|----|
|                                       | pH                 | Cond. | Temp.,<br>C | D.O.  | Alk. | Total Hard. | Susps.<br>Solids              | Diss.<br>Solids | COD (a) | TOC (b) | TKN (c) | NH <sub>4</sub> | NO <sub>2</sub>                           | NO <sub>3</sub> | PO <sub>4</sub> | SO <sub>4</sub> | C1     |        |    |
| Selected Criteria                     | 6-9                | <900  | -           | <5    | -    | -           | <80                           | <500            | <40     | <12     | <1.0    | <0.02           | -   | <1.5            | <0.1            | <250            | <250   |        |    |
| Spring                                |                    |       |             |       |      |             |                               |                 |         |         |         |                 |   |                 |                 |                 |        |        |    |
| Control (GC-1,2)                      | Mean               | 7.9   | 643         | 18.0  | 7.1  | 227         | 308                           | 232             | 476     | 35      | 7.7     | 1.9             | 0.20                                      | 0.34            | 43.7            | 0.17            | 73     | 22     |    |
|                                       | S                  | 0.27  | 103         | 0.59  | 1.11 | 35          | 45                            | 288             | 84.98   | 36.02   | 1.37    | 1.70            | 0.18                                      | 0.08            | 12.34           | 0.03            | 12.24  | 6      |    |
|                                       | Max                | 8.4   | 778         | 19.3  | 8.4  | 265         | 355                           | 638             | 598     | 88      | 9.0     | 4.7             | 0.44                                      | 0.47            | 65.0            | 0.28            | 85     | 25     |    |
|                                       | Min                | 7.5   | 470         | 16.9  | 5.4  | 165         | 249                           | 40              | 368     | 4       | 6.0     | 0.7             | <0.05                                     | 0.24            | 30.0            | 0.12            | 57     | 19     |    |
| Below Filtration Plant Outfall (GC-3) | Mean               | 7.8   | 1115        | 19.7  | 7.8  | 287         | 412                           | 394             | 999     | 36      | 9.6     | 2.0             | 0.11                                      | 0.41            | 133.4           | 0.06            | 290    | 37     |    |
|                                       | S                  | 0.55  | 288.64      | 0.61  | 0.26 | 137.81      | 66.9                          | 240.8           | 316.2   | 20.6    | 4.83    | 1.03            | 0.09                                      | 0.08            | 33.28           | 0.03            | 193.98 | 5.17   |    |
|                                       | Max                | 8.7   | 1764        | 20.2  | 8.2  | 515         | 509                           | 736             | 1446    | 64      | 18.0    | 3.4             | 0.21                                      | 0.32            | 172.0           | 0.10            | 629    | 44     |    |
|                                       | Min                | 7.3   | 753         | 18.8  | 7.6  | 143         | 333                           | 134             | 686     | 12      | 6.0     | 0.9             | <0.05                                     | 0.30            | 90.0            | 0.03            | 154    | 32     |    |
| Red Water Ditch (RW-S)                | Mean               | 7.7   | 995         | 28.7  | 6.2  | 170         | 359                           | 29              | 638     | 29      | 48      | 2.1             | 0.26                                      | 3.6             | 53              | 0.05            | 191    | 24     |    |
|                                       | S                  | 0.3   | 145         | 4.3   | 1.1  | 29          | 21                            | 24              | 49      | 5       | 10      | 0.5             | 0.34                                      | 1.8             | 17              | 0.02            | 39     | 5      |    |
|                                       | Max                | 8.2   | 1210        | 34.9  | 7.5  | 200         | 377                           | 70              | 712     | 35      | 66      | 2.4             | 0.86                                      | 5.2             | 75              | 0.08            | 258    | 25     |    |
|                                       | Min                | 7.4   | 812         | 23.3  | 4.8  | 125         | 329                           | <10             | 602     | 23      | 40      | 1.3             | <0.05                                     | 0.61            | 33              | <0.02           | 160    | 23     |    |
| Downstream (GC-4,5)                   | Mean               | 7.39  | 865         | 21.5  | 7.8  | 249         | 368                           | 258             | 627     | 34      | 8.4     | 1.5             | 0.19                                      | 1.04            | 76.2            | 0.07            | 145    | 28     |    |
|                                       | S                  | 0.39  | 177.49      | 3.23  | 1.11 | 69.95       | 45.65                         | 304.97          | 73.84   | 30.24   | 3.13    | 0.93            | 0.16                                      | 0.24            | 17.77           | 0.02            | 56.59  | 2.76   |    |
|                                       | Max                | 8.6   | 1065        | 27.5  | 9.6  | 430         | 415                           | 1008            | 730     | 112     | 14.0    | 3.8             | 0.44                                      | 1.30            | 110.0           | 0.11            | 177    | 33     |    |
|                                       | Min                | 7.2   | 585         | 18.6  | 6.4  | 161         | 279                           | 54              | 490     | 12      | 6.0     | 0.8             | <0.05                                     | 0.67            | 52.0            | 0.04            | 98     | 25     |    |
| Fall                                  |                    |       |             |       |      |             |                               |                 |         |         |         |                 |   |                 |                 |                 |        |        |    |
| Control (GC-1,2)                      | Mean               | 8.1   | 705         | 14.3  | 5.3  | 289         | 356                           | 48              | 482     | 6       | 8.5     | 1.2             | 0.13                                      | 0.03            | 3.0             | 0.13            | 77     | 19     |    |
|                                       | S                  | 0.66  | 42.59       | 2.124 | 2.71 | 18          | 26                            | 41.7            | 30      | 6.93    | 3.11    | 1.42            | 0.10                                      | 0.01            | 1.41            | 0.09            | 14.63  | 4      |    |
|                                       | Max                | 8.6   | 790         | 16.5  | 8.4  | 302         | 378                           | 92              | 512     | 16      | 13.0    | 3.4             | 0.27                                      | 0.06            | 4.0             | 0.23            | 92     | 20     |    |
|                                       | Min                | 6.3   | 650         | 13.0  | 2.2  | 264         | 323                           | <10             | 448     | <4      | 6.0     | 0.3             | 0.05                                      | <0.02           | 1.0             | 0.03            | 6.3    | 18     |    |
| Below Filtration Plant Outfall (GC-3) | High               | 8.0   | 1900        | 24.2  | 8.5  | 1290        | 794                           | 2178            | 1780    | 62      | 36.0    | 2.5             | 0.58                                      | 2.60            | 475.0           | 0.15            | 377    | 67     |    |
|                                       | Low                | 7.7   | 1100        | 18.8  | 5.2  | 196         | 372                           | 162             | 854     | 14      | 12.0    | 0.3             | 0.41                                      | 0.86            | 175.0           | 0.12            | 233    | 60     |    |
| Red Water Ditch Outfall (RW-O)        | Mean               | 8.0   | 485         | 51    | 7.5  | 64          | 19                            | 15              | 181     | 52      | 22      | 42              | 74  | 3               | 0.05            | 36              | 3.8    | 5      |    |
|                                       | S                  | 0.6   | 26          | 0.5   | 1.6  | 11          | 7.4                           | 34              | 15      | 4       | 3       | 11              | <1.0                                      | 0.02            | 15              | 1.5             | 6      | 10     |    |
|                                       | Max                | 8.4   | 510         | 60.0  | 8.0  | 85          | 35                            | 26              | 232     | 56      | 26      | 44              | 86  | 0.07            | 54              | <3              | 36     | 36     |    |
|                                       | Min                | 7.2   | 450         | 45.0  | 7.0  | 48          | 13                            | 10              | 160     | 46      | 19      | 37              | 58  | 0.03            | 19              |                 |        |        |    |
| Downstream (GC-4,5)                   | Mean               | 7.9   | 1118        | 21.7  | 8.6  | 211         | 452                           | 95              | 914     | 19.5    | 13.8    | 1.0             | 0.74                                      | 2.91            | 95.0            | 0.14            | 187    | 95     |    |
|                                       | S                  | 0.74  | 386         | 2.1   | 1.01 | 14.5        | 98                            | 23              | 265     | 1.0     | 2.63    | 0.24            | 0.32                                      | 1.52            | 48.29           | 0.08            | 10.34  | 115.67 | 4  |
|                                       | Max                | 8.4   | 2200        | 25.3  | 9.9  | 224         | 586                           | 116             | 1266    | 20      | 16.0    | 1.2             | 1.00                                      | 4.10            | 160.0           | 0.22            | 196    | 268    | 36 |
|                                       | Min                | 7.2   | 900         | 19.0  | 6.9  | 190         | 372                           | 64              | 670     | 18      | 11.0    | 0.7             | 0.27                                      | 0.74            | 46.0            | 0.04            | 173    | 36     |    |

(a) Chemical Oxygen Demand

(b) Total Organic Carbon

(c) Total Kjeldahl Nitrogen

A small drainage system, designated as the red water ditch, which collects both runoff and process area discharges, empties into Grant Creek between stations GC-3 and GC-4. These process waste constituents and degradation products are believed to be responsible for the unusual nitrogen species concentrations, most pronounced in the fall sampling. The red water industrial discharge (RW-0) also had high chemical oxygen demand. A mean of 154 mg/l with low variability was reported from the fall sampling period. An average discharge temperature of over 51 degrees C (fall survey) is excessive for most aquatic life. This elevated temperature, however, was not reflected in increased temperatures in Grant Creek.

The water quality data for stations GC-4 and GC-5 have been composited (Table 3). The discharges from the red water drainage system and treatment plant above GC-4 serve to dilute inorganic constituents in Grant Creek. However, increases in nitrogen species concentrations contribute to nutrient enriched conditions downstream. On occasion, the increased chemical oxygen demand (COD) values in this discharge may also result in undesirable conditions.

Review of the data in Appendix A, Table 1, showed the influence of an early morning rainstorm (6/17/75) on the water quality of the JAAP stations. In Grant Creek increased suspended matter and decreased dissolved matter were apparent for that sampling date. These values, however, do not greatly alter the overall water chemistry parameters summarized in Table 3. In general, water quality characteristics in Grant Creek are being detrimentally altered by both JAAP effluents.

#### Sediment Chemistry

A summary of the combined analyses of the sediment characteristics from both surveys appears in Table 4. Values defining lightly and heavily "polluted" sediment characteristics are also given. Results of all analyses of sediments from spring and fall periods are presented in Appendix B, Tables 1 and 2.

TABLE 4. SUMMARY OF SEDIMENT CHARACTERISTICS FOR GRANT CREEK STATIONS, SPRING AND FALL, 1975

|   | Total Solids                    | Volatile Solids | Chemical Oxygen Demand | Total Kjeldahl Nitrogen     | Total Phosphate     | Number of Samples |
|---|---------------------------------|-----------------|------------------------|-----------------------------|---------------------|-------------------|
|   | (Values in percent, dry weight) |                 |                        | (Values in ppm, dry weight) |                     |                   |
| Objectionable <sup>(a)</sup><br>Sediment<br>Characteristics | >6.0                            |                 | >5.0                   | >1000                       |                     |                   |
| Polluted Sediments<br>"Light" <sup>(b)</sup>                | <5.0                            |                 | <4.0                   |                             | <300 <sup>(c)</sup> |                   |
| "Heavy"   | >8.0                            |                 | >12.0                  |                             | >900                |                   |
| <u>Grant Creek</u>  |                                 |                 |                        |                             |                     |                   |
| Upstream<br>(GC-1&2)  | 78.9                            | 1.63            | -                      | 104                         | -                   | 2                 |
|   | 75.8                            | 1.33            | -                      | 55                          | -                   |                   |
| Downstream<br>(GC-3,4,5)                                    |                                 |                 |                        |                             |                     |                   |
| Mean  | 75.1                            | 1.33            | 0.72                   | 112                         | 240                 | 13                |
| S   | 14.5                            | 0.41            | 0.32                   | 50                          | 212                 |                   |
| Max   | 89.8                            | 2.05            | 1.11                   | 199                         | 652                 |                   |
| Min   | 74.4                            | 0.83            | 0.31                   | 67                          | 28                  |                   |

(a) Selected bulk analysis allowable sediment constituents (National Science Foundation, 1973)

(b) Selected bulk analysis classification of polluted sediments (Corps of Engineers, 1970).

(c) Originally reported as P.

The COD values of the sediments in Grant Creek are not considered excessively high. Phosphate concentrations, on occasion, were somewhat elevated downbasin. It is possible that the highly flocculent, dominantly inorganic, bottom sediments which are discharged from the water filtration plant dilute the characteristics of this stream's bed load. In any case, bed load impacts on the Des Plaine River [already of secondarily-treated sewage nature (Battelle Columbus Laboratories, 1972; U. S. Environmental Protection Agency, 1974)] are expected to be immeasurable.

Grant Creek Munitions Constituent Analysis

Water. Grant Creek water samples analyzed from the spring sampling period at JAAP indicated that stream concentrations of the nitrotoluene munitions were generally low, on the order of about 5 - 50 ppb. Concentrations of TNT and two degradation products are summarized in Table 5. When very low concentrations of these munitions are being measured, large fluctuations in the data might be anticipated, as small amounts of material can cause relatively large changes in concentrations. The objective was to analyze for dissolved munitions, but the samples were not filtered and some heterogeneity may have occurred.

Munitions concentrations in the effluent at the outfall (RW-0) were considerably higher than stream values. Redwater stream (RW-S) concentrations usually ranged between 20 - 50 ppb with a value of 133 ppb determined for TNT in one sample, indicating an abnormally high discharge on that day (Appendix A, Table 1). Munitions concentrations in GC-4 water samples averaged somewhat lower than those at RW-S. GC-5 water values were even lower, being consistently below 16 ppb for all three compounds monitored. This general trend indicates that the concentrations of the munitions in the water phase were rapidly diminished along the route of the waste stream, possibly due either to degradation or the settling out of the materials into the sediment.

TABLE 5. MEAN CONCENTRATIONS (RANGES) OF TNT, 2,4 DNT AND 2,6 DNT IN SELECTED WATER SAMPLES FROM GRANT CREEK STATIONS.

|                     | TNT (ppb)       | 2,4 DNT (ppb)   | 2,6 DNT (ppb)  |
|---------------------|-----------------|-----------------|----------------|
| <b>Spring, 1975</b> |                 |                 |                |
| RW-0                | 75 (4.2-145.7)  | 258 (7.2-509.7) | 85 (2.9-167.3) |
| RW-S                | 55 (24.8-133.2) | 16 (12.4-21.2)  | 24 (12.1-62.7) |
| GC-4                | 25 (7.6-63.7)   | 12 (2.2-43.6)   | 28 (4.6-49.5)  |
| GC-5                | 9 (4.0-12.4)    | 10 (6.0-15.9)   | 13 (13.0-14.0) |
| <b>Fall, 1975</b>   |                 |                 |                |
| RW-0                | 23 (19.4-24.8)  | 63 (55.0-67.7)  | 20 (18.1-22.1) |
| GC-4                | 30 (26.7-32.5)  | 19 (18.9-19.5)  | 15 (13.5-15.8) |
| GC-5                | 28 (18.0-37.7)  | 17 (11.8-21.8)  | 15 (12.6-16.8) |

Fairly low levels of munitions concentrations for the production facility waste stream were indicated in fall samples, as might be anticipated as a reduction in the operation of this facility had occurred. Water samples showed small fluctuations in concentrations, with values generally ranging less than 30 ppb for all munitions examined in the water at stations downstream from the production facility outfall (Appendix A, Table 2).

The two DNT isomers (2,6 and 2,4 - dinitrotoluene) were also present in these water samples. The DNT concentrations were similar to those found in the spring samples. Values were more consistent in the fall samples producing slightly higher means for the 2,4 isomer at GC-4 than calculated for spring samples.

Results of all munitions compounds analyses of water samples from spring and fall sampling periods appear in Appendix A, Tables 1 and 2, respectively.

Sediment. Sediment samples characteristically contained significant levels of materials which activated the electron capture detector. Hence, actual concentrations may vary within 2-3 ppb of the values reported. The data collected on JAAP sediment samples from the spring survey indicated that, while the munitions concentrations were still less than 1 ppm, some accumulation did occur in the sediment as a result of the settling-out of the materials from the water phase (Table 6). Concentrations of TNT and DNT appeared to fluctuate widely on a daily basis. TNT concentration levels in GC-4 and GC-5 sediment samples on some days ranged to several hundred ppb, while values equivalent to the water concentrations were observed on other days. Mean munitions concentrations in the spring sediment samples were actually higher at GC-5 than at GC-4. However, sediment samples from the fall sampling period exhibited fairly static conditions. The accumulation of TNT in the GC-4 sediments was on the order of a few hundred ppb, which sharply fell at the GC-5 station, to about the same levels as found in the corresponding water samples.

DNT levels in sediment samples were generally somewhat higher than TNT values during both sampling periods, and appeared to follow the trends displayed by the TNT concentrations. However, 2,4 DNT concentrations through the two sampling periods averaged to 369 ppb, while 2,6 DNT levels averaged to 240 ppb, perhaps reflective of the lower stability of 2,6 DNT to photolytic decomposition.

TABLE 6. MEAN CONCENTRATIONS (RANGES) OF TNT, 2,4 DNT  
AND 2,6 DNT IN SEDIMENT SAMPLES FROM SELECTED  
GRANT CREEK STATIONS

|                     | TNT (ppb)         | 2,4 DNT (ppb)     | 2,6 DNT (ppb)     |
|---------------------|-------------------|-------------------|-------------------|
| <b>Spring, 1975</b> |                   |                   |                   |
| GC-4                | 68 (10.1-165.0)   | 278 (58.5-632.5)  | 176 (32.3-426.0)  |
| GC-5                | 205 (29.2-363.5)  | 598 (55.4-1203.8) | 437 (58.5-738.0)  |
| <b>Fall, 1975</b>   |                   |                   |                   |
| GC-4                | 170 (116.6-233.5) | 458 (293.0-560.7) | 265 (212.6-327.7) |
| GC-5                | 50 (43.2-61.5)    | 142 (120.2-166.5) | 83 (77.5-90.5)    |

Results of all munitions compounds analyses of sediment samples for spring and fall sampling surveys are presented in Appendix B, Tables 1 and 2, respectively.

Several unidentified species were observed in these water and sediment samples which were capable of activating the electron capture detector. Four such species were routinely observed in the chromatograms of these samples with retention times of 2.6, 3.3, 3.7, and 4.3 minutes, as shown in Figure 4. Although not quantitated, their concentrations appeared to be on the order of a few ppb. The peak occurring at 3.7 minutes was generally the most prominent of these unidentified peaks, being the only one of the four unknown species observed in the Doyle Lake (DL) water samples, but generally not observed in any outfall samples. The presence of the peak at 3.3 minutes introduces a source of error in the determination of low concentrations of 2,4 - DNT in DL water samples. The concentrations of these four species in sediment samples appeared to be of the same order of magnitude as that found in water samples.

The widely fluctuating concentrations of nitrotoluenes in the water and sediment samples examined may be attributable to intermittent discharges coupled with a fairly rapid process by which these munitions were migrating and/or degrading, as might be experienced in shallow, rapidly flowing streams.

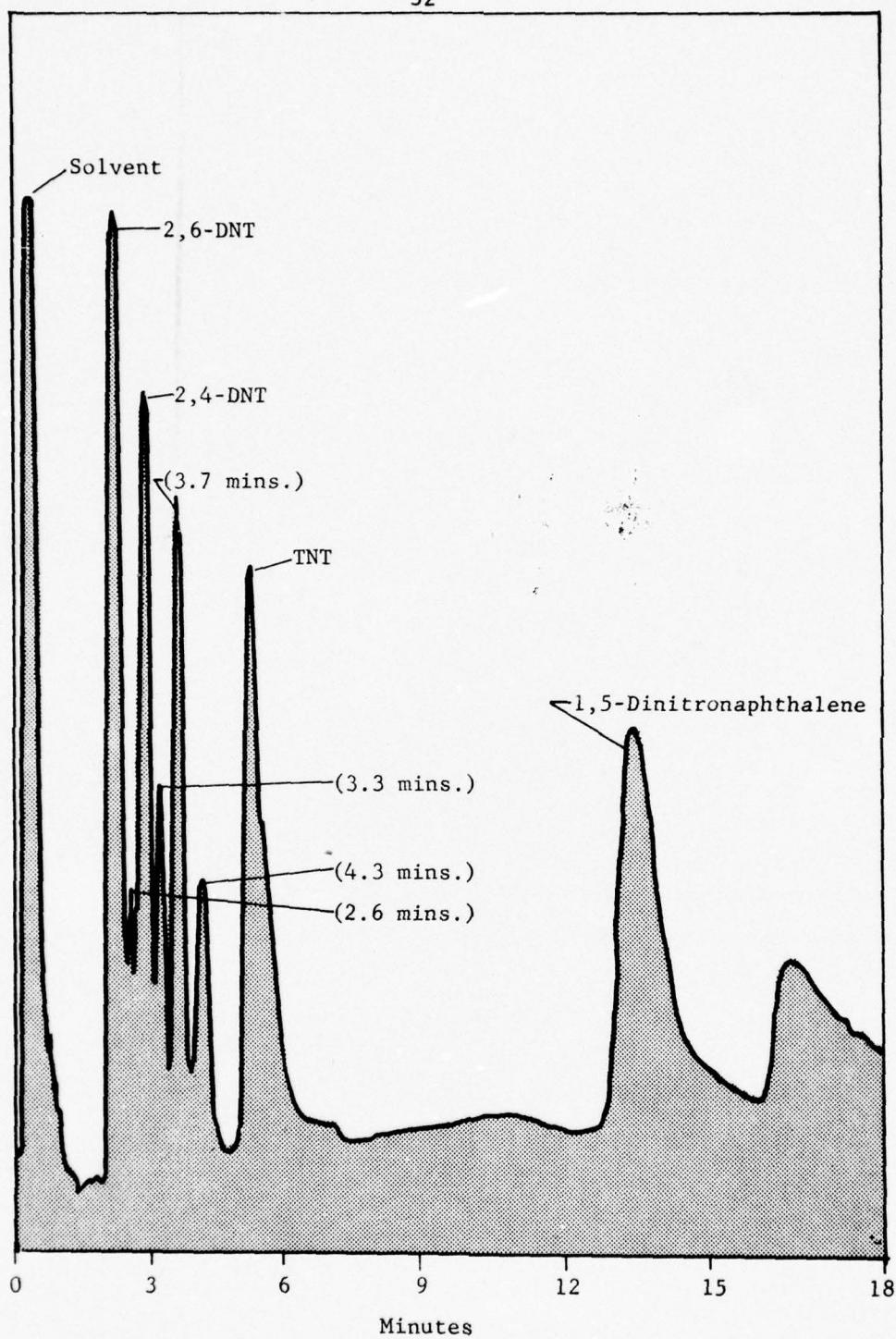


FIGURE 4. CHROMATOGRAM OF GC-5 JUNE 16, 1975, WATER SAMPLE EXTRACT

Water concentrations rapidly declined through a downstream sequence of stations while the sediment values varied widely, indicating possible deposition of munitions in the sediment.

#### Grant Creek Periphyton (Spring)

The physical-chemical characteristics of Grant Creek during spring sampling were largely influenced by spring rains which created high flows in this small headwater stream. This high flow enabled samples to be collected from intermittent portions of Grant Creek (GC-1). It also completely flushed the stream of the flocculant substance contained in the water treatment plant effluent which often covers the bottom substrates below GC-3. Stream conditions in the spring reduced the relative impact of this discharge and allowed a more accurate assessment of the effect of the discharge of "pink water" above GC-4.

Species Composition. In spite of the diluting capacity of the high water levels in Grant Creek during the spring survey, alterations occurred in the periphyton species compositions below the JAAP effluents. Large populations of Achnanthes lanceolata, A. linearis, and Amphora ovalis var. pediculus became dominant at GC-3, immediately below the filtration plant effluent. At GC-4 and GC-5, below the pink water outfall, these same species shared dominance with several very tolerant Navicula and Nitzschia species (Palmer, 1969) and Surirella ovalis, (Appendix C, Tables 3-5). Numbers of species remained fairly constant between stations. Unidentified coccoid green and blue-green algal species and unicellular flagellates were consistently dominant at all stations.

Dominant species colonizing the diatometers during the three-week incubation period were generally comparable to those abundant on natural substrates (Appendix C, Tables 7-11). However, due to the loss of the diatometer at GC-4 the amount of data for this station was greatly reduced, hence, the shift to tolerant species below the plant outfalls is not so pronounced as on the natural substrates.

Two species were found to exist exclusively at stations upstream of the pink water outfall--Gomphonema tergestinum, occurring only occasionally and Cocconeis placentula var. euglypta, which occurred occasionally on natural

substrates but was a dominant species on diatometer slides. The latter is considered a clean water species, but the status of this variety is undetermined (Palmer, 1957 and 1959).

Summary tables of species found on natural and artificial substrates appear in Appendix C, Tables 6 and 12, respectively.

Community Structure. Review of the mean numbers of individuals and species and mean species diversities (Table 7) showed little variation among algal communities found on natural substrates in Grant Creek during the spring survey. There was an increase in the number of individuals at GC-4 and GC-5 with a corresponding slight increase in the number of species. The overall effect of these increases, however, was not expressed as a significant increase in species diversity.

TABLE 7. MEANS OF NATURAL SUBSTRATE PERIPHYTON DATA  
FROM GRANT CREEK, SPRING, 1975

|      | Mean Number of<br>Individuals $\times 10^4$ | Mean Number of<br>Species | Species Diversity |
|------|---|---------------------------|-------------------|
| GC-1 | 24.7  | 11.2                      | 1.13              |
| GC-2 | 16.4  | 8.6                       | .98               |
| GC-3 | 18.5  | 11.4                      | 1.44              |
| GC-4 | 35.5  | 13.2                      | 1.73              |
| GC-5 | 36.0  | 13.2                      | 1.39              |

ANOVA of Grant Creek Periphyton. Table 8 presents the results of analyses of variance performed on natural substrate periphyton data to test for differences among all five Grant Creek Stations.

TABLE 8. ANOVA OF NATURAL SUBSTRATE PERIPHYTON DATA FROM GRANT CREEK, SPRING, 1975

| Site | No. of Individuals X10 <sup>5</sup> | Site | No. of Species | Site | $\bar{H}$ |
|------|-------------------------------------|------|----------------|------|-----------|
| GC-2 | 1.6                                 | GC-2 | 8.6            | GC-2 | 0.98      |
| GC-3 | 1.8                                 | GC-1 | 11.2           | GC-1 | 1.13      |
| GC-1 | 2.4                                 | GC-3 | 11.4           | GC-5 | 1.39      |
| GC-4 | 3.5                                 | GC-4 | 13.2           | GC-3 | 1.44      |
| GC-5 | 3.6                                 | GC-5 | 13.2           | GC-4 | 1.72      |

Significance .90  
level of F-test

— no significant difference

The variables analyzed for significant differences were total number of individuals, total number of species, and diversity index ( $\bar{H}$ ). In Table 8, each pair of adjacent columns presents the results of one Duncan's Mean Separation Test which accompanied the corresponding ANOVA; the significance level is stated below the second column in the pair. The mean separation lines which portray the differences and similarities of the means are drawn vertically to the immediate right of those means. If any line segment connects two means, those two means are similar, whereas they are significantly different if no line segment connects both of them.

The ANOVA on natural substrate periphyton showed the mean numbers of individuals at GC-4 and GC-5 to be higher than at all the other Grant Creek stations. Differences in the mean number of species and in the mean diversity index were not significant, but numbers of species at GC-4 and GC-5 again were higher than at all other sampling stations.

Figure 5 is a graphic representation of the status of the algal community at each station in Grant Creek. Curves were plotted from the cumulative numbers of species found on natural substrates versus the area sampled. Study of these curves shows that the number of analyses performed was sufficient to describe each community. The similarity between curves and the narrow range of the end points (23-30) indicate slight, if any, difference between communities.

The proportion of algal groups on natural substrates appeared fairly constant at stations GC-1 and GC-2 (Table 9). Flagellates dominated at all stations. Diatoms began to increase in number at station GC-3 and maintained large populations downstream while the percentage of green algae species steadily declined. Such fluctuations in the populations of groups of organisms indicates a perturbation to their normal or optimum conditions (Patrick and Strawbridge, 1963). In the case of Grant Creek, a perturbation is indicated at the confluence of the filtration plant outfall (GC-3) and again below the pink water outfall (GC-4).

Periphytic community structures on artificial substrates, while maintaining fairly constant numbers of species, showed large shifts in population sizes below both plant outfalls. During the first week of incubation, populations of flagellates increased dramatically at both stations GC-3 and GC-4. However, these organisms were greatly reduced in numbers at the second week causing the total number of individuals at GC-3 to drop (Table 10). Again, loss of the diatomometer at GC-4 prevents comparison with the periphyton community response to the pink water outfall at GC-4 during the second or third weeks.

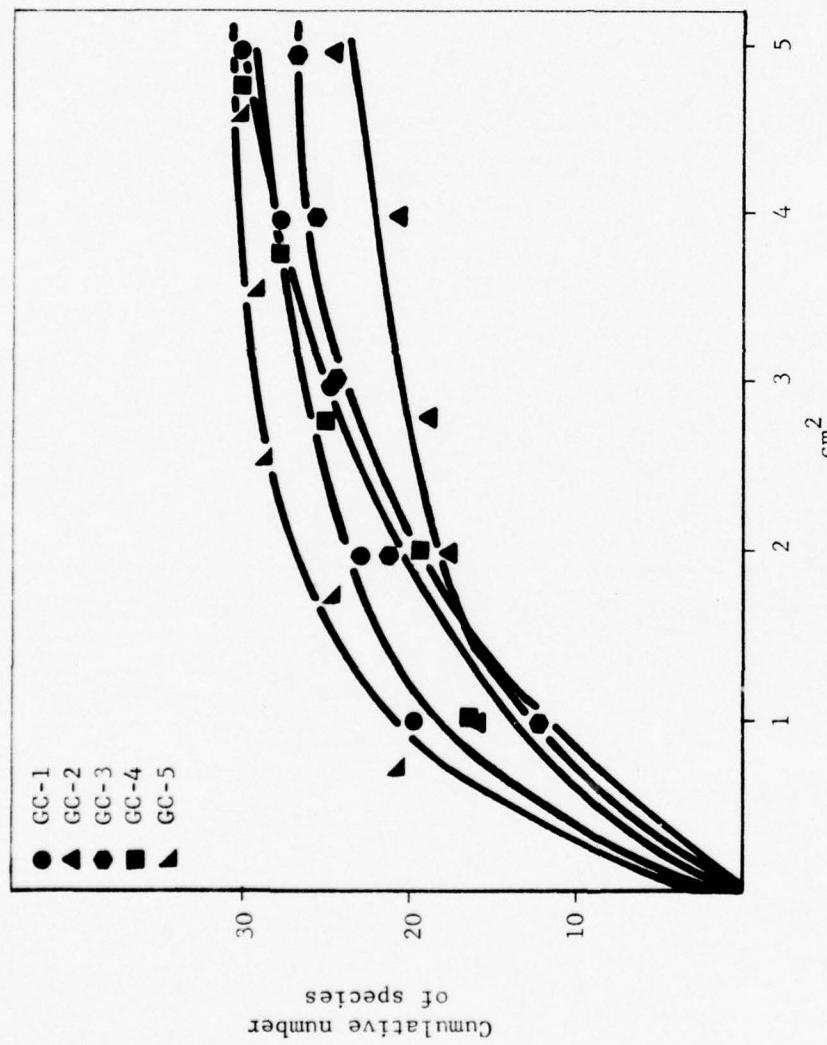


FIGURE 5. SPECIES-AREA CURVES FROM GRANT CREEK PERIPHYTON DATA, SPRING, 1975

TABLE 9. PROPORTIONS OF NATURALLY OCCURRING PERIPHYTON GROUPS  
AT GRANT CREEK STATIONS, SPRING, 1975

|      | Chrysophyta<br>(Diatoms) | Chlorophyta<br>(Greens) | Cyanophyta<br>(Blue-green) | Flagellates |
|------|--------------------------|-------------------------|----------------------------|-------------|
| GC-1 | 0.06                     | 0.38                    | 0.22                       | 0.33        |
| GC-2 | 0.04                     | 0.36                    | 0.21                       | 0.38        |
| GC-3 | 0.19                     | 0.31                    | 0.18                       | 0.31        |
| GC-4 | 0.38                     | 0.18                    | 0.15                       | 0.28        |
| GC-5 | 0.28                     | 0.14                    | 0.13                       | 0.45        |

TABLE 10. MEANS OF ARTIFICIAL SUBSTRATE PERIPHYTON DATA  
FROM GRANT CREEK, SPRING, 1975

|      | Week 1                                      | Week 2                                      | Week 3                                      |                           |
|------|---|---|---|---------------------------|
|      | Mean Number of<br>Individuals $\times 10^4$ | Mean Number of<br>Individuals $\times 10^4$ | Mean Number of<br>Individuals $\times 10^4$ |                           |
|      | Mean Number of<br>Species                   | Mean Species<br>Diversity                   | Mean Number of<br>Species                   | Mean Number of<br>Species |
|      |   |   |   | Mean Species<br>Diversity |
| GC-1 | 1.8   | 13  | 1.95  | 6.0                       |
| GC-2 | 1.7   | 12  | 1.57  | 4.8                       |
| GC-3 | 4.2   | 15  | 1.29  | 2.7                       |
| GC-4 | 10.5  | 13  | 1.80  | --                        |
| GC-5 | 1.4   | 12  | 1.79  | 6.7                       |

Only those samples taken during the intensive sampling week were considered in the analysis of variance of the artificial substrate data. (Only one replicate for each station from weeks 1 and 3 was analyzed in the laboratory, yielding insufficient data for this type of statistical analysis). The results of the ANOVA's are presented in Table 11.

TABLE 11. ANOVA OF ARTIFICIAL SUBSTRATE PERIPHYTON DATA  
FROM GRANT CREEK, SPRING, 1975

| Station                          | Number of IndividualsX10 <sup>4</sup> | Station | Number of Species | Station | $\bar{H}$ |
|----------------------------------|---------------------------------------|---------|-------------------|---------|-----------|
| GC-3                             | 2.71                                  | GC-2    | 15.3              | GC-5    | 1.90      |
| GC-2                             | 4.8                                   | GC-3    | 15.6              | GC-2    | 2.04      |
| GC-1                             | 6.0                                   | GC-5    | 15.6              | GC-1    | 2.19      |
| GC-5*                            | 6.7                                   | GC-1    | 21.0              | GC-3    | 2.20      |
| Significance level of F-test .99 |                                       |         | .98               |         | —         |

\* diatomometer lost at GC-4  
— no significant difference

The artificial substrate periphyton showed a significantly lower mean number of individuals at GC-3 than at all other stations. The mean number of species was significantly higher at GC-1 than at all other stations; the mean diversity index did not differ significantly among the sampling stations. At GC-3 there did not appear to be any notable shifts in species present nor in the relative proportions of individuals within those species; the decrease in community size was caused by equal reductions in the population sizes of all species.

GC-4 and GC-5 had the highest standing crops on both natural and artificial substrates. High species diversities were also found at these stations indicating a nutrient enrichment condition affecting all species equally.

The ANOVA do not indicate any adverse impact caused by the TNT effluent upon the periphyton communities. However, there may have been a slight impact caused by the water treatment plant outfall upon the periphyton found on artificial substrate samples at GC-3.

Biomass, Chlorophyll a, and Autotrophic Index. Ash-free, dry-weight biomass and chlorophyll a determinations for stations in Grant Creek are summarized in Table 12. In comparison to the amount of biomass present, chlorophyll a content was very low. Such a condition suggests that much of the material in Grant Creek is not autotrophic. Review of the species collected from Grant Creek (Appendix C, Tables 7-11), show large numbers of single-celled flagellates contributing heavily to the biomass. These organisms may or may not contain chlorophyll a. Bacteria, fungi, and/or dead algal masses may also be contributing nonchlorophyll-containing biomass. The autotrophic indices calculated from the biomass and chlorophyll a data are all above 100. Weber and McFarland (1969), have set this value as the lower limit in describing systems receiving organic pollution.

TABLE 12. BIOMASS, CHLOROPHYLL a, AND AUTOTROPHIC INDICES FOR GRANT CREEK, SPRING, 1975

|      | Biomass<br>mg/m <sup>2</sup> | Chlorophyll <u>a</u><br>mg/m <sup>2</sup> | Autotrophic Index<br>Biomass/Chl <u>a</u> |
|------|------------------------------|---|---|
| GC-1 | 1390.7                       | 9.55                                      | 145.6                                     |
| GC-2 | 805.1                        | 6.35                                      | 126.9                                     |
| GC-3 | 1321.8                       | 2.94                                      | 449.3                                     |
| GC-4 | —                            | —   | —   |
| GC-5 | 1442.4                       | 11.07                                     | 130.5                                     |

Colonization. Numbers of individuals colonizing the artificial substrate were observed to steadily increase throughout the three week incubation period (Table 10). Most species and highest diversities occurred at all stations during the second week, a common interval for peak communities colonizing artificial samplers (Patrick, 1957; Dickson, personal communication). Increased numbers of individuals and lowered diversities found during the third week were caused by population increases of only two or three competitive species at each station (Appendix C, Tables 7-11).

Figure 6 presents dendograms of cluster analyses on periphyton data from all five Grant Creek stations, for natural (A) and artificial (B) substrate samples.

The dendogram from the natural substrate data was formed by the following steps: (1) the two downstream stations GC-4 and GC-5 merged at a level of 0.25, (2) all three upstream stations merged at a level of only 0.19, and (3) the cluster for the upstream stations merged with the cluster for the downstream stations at a level of 0.17.

The dendrogram of the data from artificial substrates was formed by these steps: (1) the two upstream stations GC-1 and GC-2 merged at a level of 0.26, (2) GC-3 merged with the cluster for GC-1 and GC-2 at a level of 0.23, and (3) GC-5 merged with the cluster for all upstream stations at a level of 0.20. GC-4 was dissimilar to all other stations as its data resulted from only one replicate; it has therefore been eliminated from consideration in this discussion.

Both dendograms indicate only a subtle difference in species assemblages between the upstream and downstream stations. The artificial substrate dendrogram also indicated a very slight difference between GC-3 and the stations upstream of the water treatment plant outfall.

#### Grant Creek Periphyton (Fall)

Species Composition. Analysis of the natural substrate periphyton samples taken during the fall survey showed the two stations (GC-1 and GC-2) upstream of plant effluents to have the most diverse algal communities. A total of 64 species were found at these two stations. Several species shared dominance including the clean-water species Amphora ovalis (Palmer, 1959). Two other species intolerant of pollution were also present at these stations (Cocconeis placentula and Nitzschia linearis).

A large reduction in the number of algal species occurred immediately below the water treatment plant outfall (36 species at GC-3). The numbers of tolerant blue-green algae (Cyanophyta) species increased, particularly those of Microcystis (Palmer, 1969). At stations GC-4 and GC-5, algal communities were dominated by large numbers of pollution resistant algae - Navicula cryptocephala,

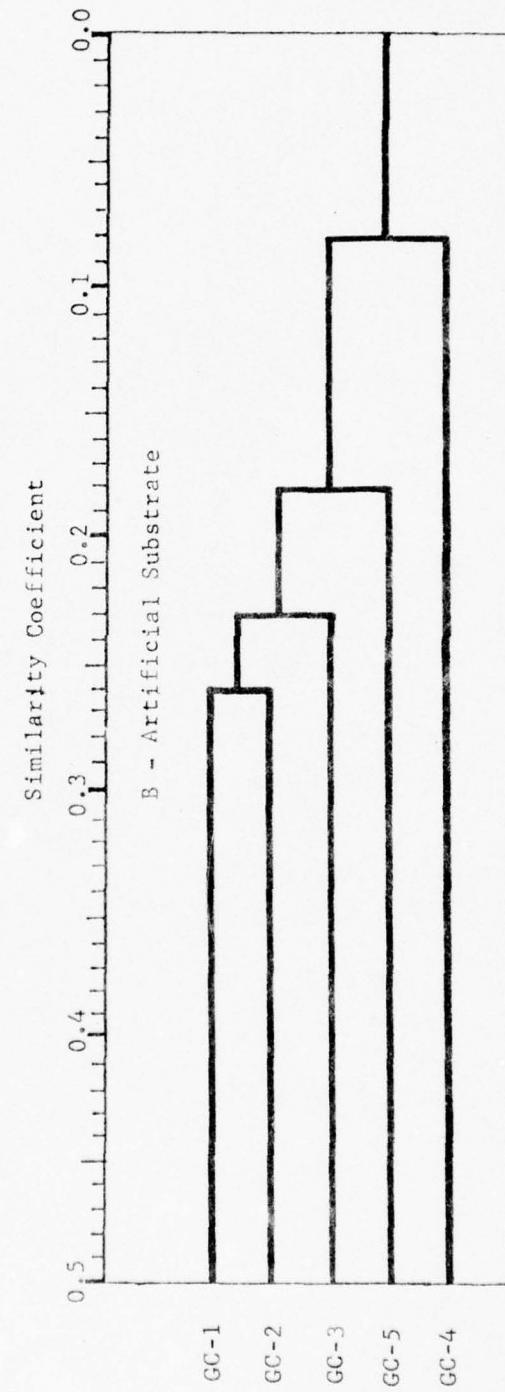
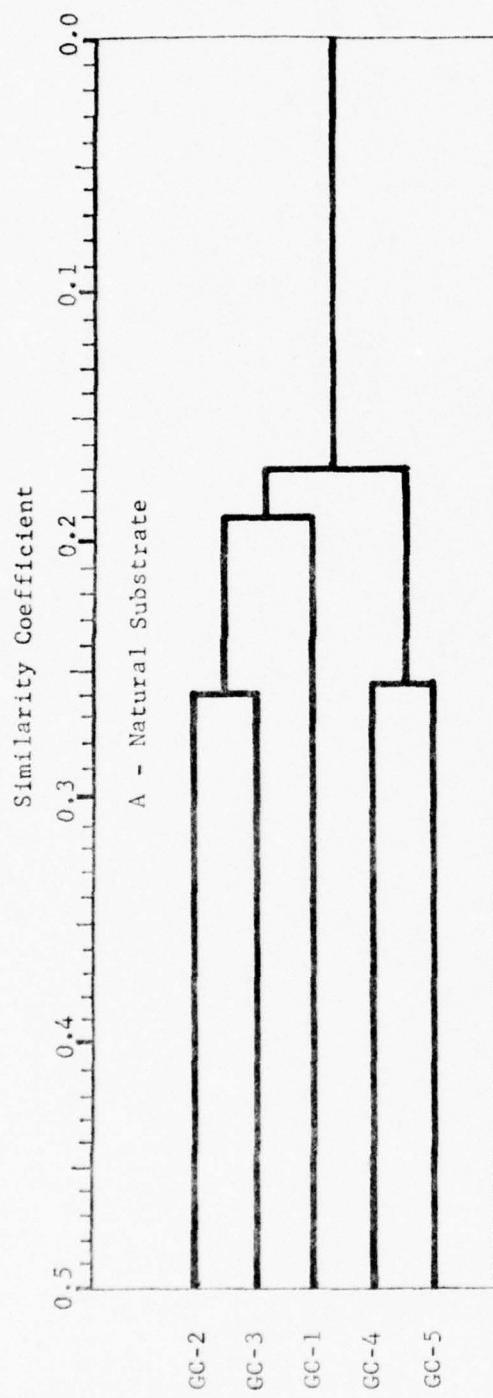


FIGURE 6. DENDROGRAM OF GRANT CREEK PERiphyton DATA COLLECTED FROM NATURAL (A) AND ARTIFICIAL (B) SUBSTRATES, SPRING, 1975

Nitzschia amphibia, N. palea, Scenedesmus quadricauda (Palmer, 1959).

Total numbers of species increased between GC-3 and GC-4 but had not yet reached upstream numbers. The composition had also shifted from relatively clean-water species upstream to predominantly pollution-tolerant species below the plant's pink water outfall.

Similar patterns were observed in the data from artificial substrate samples. Eighty-four species were collected upstream. Dominance was divided among several tolerant and clean-water species, with no species exhibiting over-abundance. At GC-3, the number of species had dropped to 37, with an increase in tolerant blue-green species. Some recovery in number of species (58) occurred at GC-4 and GC-5, but again the dominance had shifted to very tolerant organisms - Navicula cryptocephala, Nitzschia amphibia, N. palea, in particular.

On artificial substrates, fourteen species, which had occurred in more than one sample at each upstream station, were not found downstream of the pink water outfall in Grant Creek. Similarly, 22 species occurring on natural substrates upstream were not found downstream. Of those species, Amphipleura pellucida, Cocconeis placentula, Cymatopleura solea, Gomphonema acuminatum, Gyrosigma scalproides, and Stauroneis Smithii were found upstream but not downstream on both natural and artificial substrates.

Data sets of fall periphyton investigations are presented in Appendix C, Tables 13-24.

Community Structure. An abrupt alteration in the community structure of the natural substrate periphyton of Grant Creek was observed immediately below the JAAP pink water outfall. Species diversity dropped slightly at GC-3, below the water treatment plant but decreased significantly over upstream values at GC-4. (Table 13 presents the mean numbers of individuals and species and species diversities for Grant Creek). No change in the mean number of species

occurred between stations. However, the drop in species diversity can be attributed to large increases in several extremely tolerant organisms at GC-4 - Navicula cryptocephala, N. minima, Nitzschia amphibia, N. fonticola, N. palea, and a coccoid blue-green species, in particular. This extreme abundance of a few species denotes a stressed environmental condition (Patrick and Strawbridge, 1963).

TABLE 13. MEANS OF NATURAL SUBSTRATE PERIPHYTON DATA FROM GRANT CREEK, FALL, 1975

|      | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity |
|------|--|------------------------|------------------------|
| GC-1 | 70.2                                     | 23.6                   | 2.52                   |
| GC-2 | 32.8                                     | 17.6                   | 2.15                   |
| GC-3 | 32.5                                     | 15.8                   | 2.02                   |
| GC-4 | 378.1                                    | 18.8                   | 1.65                   |
| GC-5 | 145.5                                    | 17.2                   | 1.61                   |

The above observations are further supported by the ANOVA performed on the natural substrate periphyton data (Table 14). The mean numbers of individuals were highest at GC-4 and GC-5, with GC-4 in particular having a significantly higher mean than all other stations. Yet, species diversities were significantly lower at GC-4 and GC-5 than at stations above the pink water effluent. The lowest mean number of individuals and species occurred at GC-3, but the difference was not significant when compared with upstream stations. The data here indicates a greater perturbation on the periphyton community structure below the pink water outfall than at the water treatment outfall.

TABLE 14. ANOVA OF NATURAL SUBSTRATE PERIPHYTON DATA  
FROM GRANT CREEK, FALL, 1975

| Station | Number of<br>Individuals $\times 10^5$ | Station | Number of<br>Species | Station | $\bar{H}$ |
|---------|--|---------|----------------------|---------|-----------|
| GC-3    | 3.3                                    | GC-3    | 15.8                 | GC-5    | 1.61      |
| GC-2    | 3.3                                    | GC-5    | 17.2                 | GC-4    | 1.65      |
| GC-1    | 7.0                                    | GC-2    | 17.6                 | GC-3    | 2.02      |
| GC-5    | 14.5                                   | GC-4    | 18.8                 | GC-2    | 2.15      |
| GC-4    | 37.8                                   | GC-1    | 23.6                 | GC-1    | 2.52      |

Significance  
level of F-test >.999

---

— no significant difference

Results of an analysis of the periphyton community composition by proportion of algal groups at each station masks the effects of the outfalls, particularly the pink water effluent to Grant Creek. Table 15 presents the proportion of algal groups found at each station in Grant Creek. Community composition was seen to change between GC-1 and GC-2. At GC-2, the green algae species (Kirchneriella and a coccoid species) as well as a coccoid blue-green species were most numerous. The percentage of blue-greens was increased at GC-3 in the form of a large growth of Microcystis. The percentage of algal groups returned to approximate upstream proportions below the pink water outfall at GC-4 and GC-5.

TABLE 15. PROPORTIONS OF NATURALLY-OCCURRING PERiphyton GROUPS AT GRANT CREEK, FALL, 1975

|      | Chrysophyta<br>(Diatoms) | Chloraphyta<br>(Greens) | Cyanophyta<br>(Blue Greens) | Flagellates | Other |
|------|--------------------------|-------------------------|-----------------------------|-------------|-------|
| GC-1 | .85                      | .07                     | .05                         | .02         | --    |
| GC-2 | .34                      | .29                     | .23                         | .09         | .05   |
| GC-3 | .46                      | .09                     | .34                         | .09         | .02   |
| GC-4 | .91                      | .06                     | .01                         | .01         | .001  |
| GC-5 | .87                      | .06                     | .05                         | .02         | --    |

Figure 7 is a graphic representation of the status of the periphyton community at each station in Grant Creek. Extrapolation indicates the approximate number of species to be found with further analysis. With the possible exception of GC-1, all communities appear to be adequately defined. GC-1 has consistently been shown to reflect the most diverse community sampled at Grant Creek. Further analysis would serve to improve its status and was therefore judged to be unnecessary.

Unlike the data from the natural substrates, data obtained from the diatometers showed the greatest perturbation to the periphyton communities immediately below the water treatment outfall at GC-3, rather than below the pink water outfall. Table 16, presenting means of the measured community parameters, shows consistent reductions at GC-3 during each of the 3 weeks of incubation. Dominant species were of the tolerant variety, yet no strong dominance was exhibited by any one species. Conditions at this station were altered by the flocculant material in the water treatment effluent. This heavy suspension may have caused scouring of the diatometer slides resulting in reduced populations. Communities downstream of the pink water outfall showed high diversities comparable to upstream values. However,

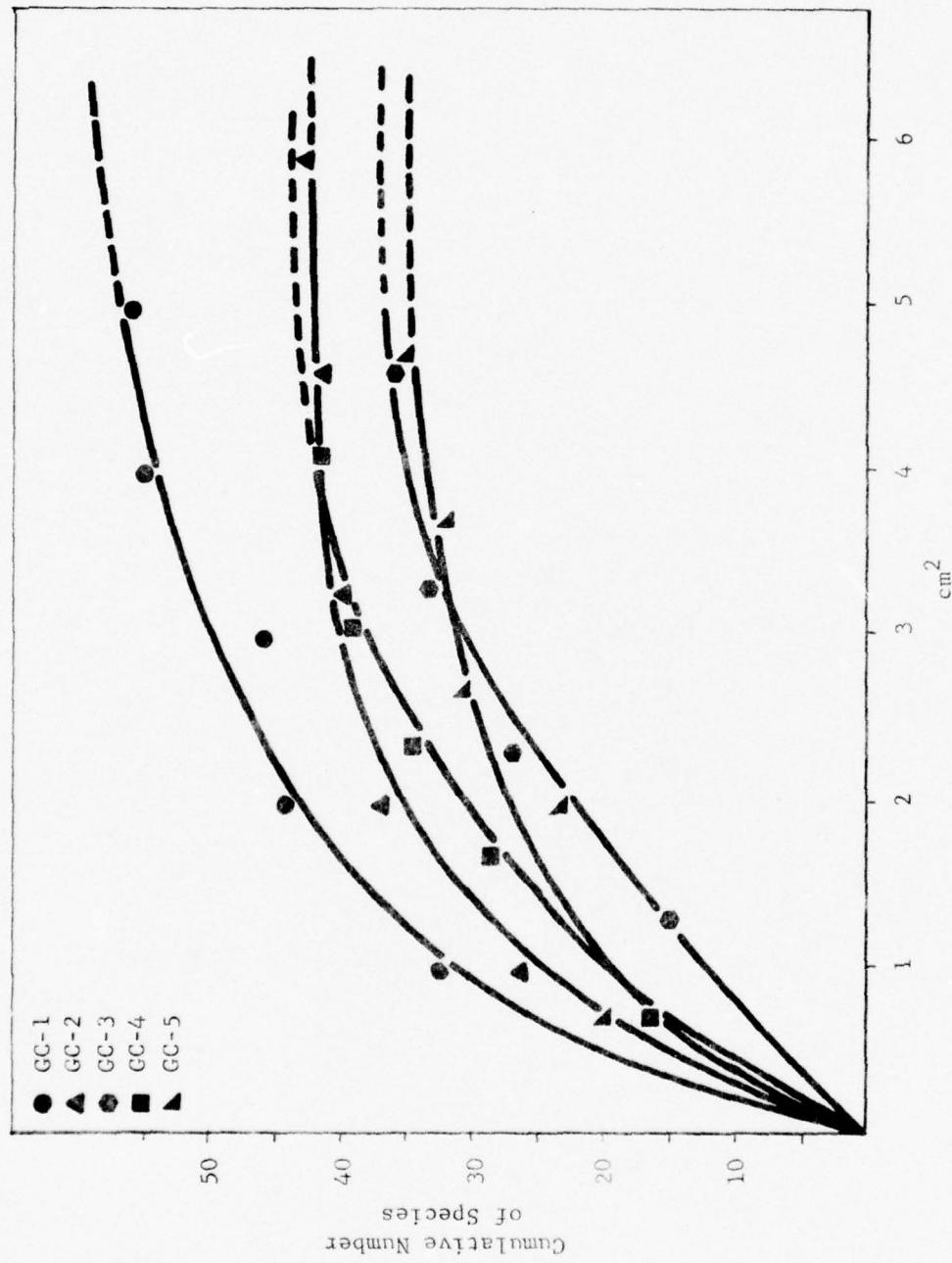


FIGURE 7. SPECIES-AREA CURVES FROM GRANT CREEK PERIPHYTON DATA (NATURAL SUBSTRATES), FALL, 1975

these downstream communities maintained no populations of clean water species and were comprised predominantly of tolerant forms.

TABLE 16. MEANS OF ARTIFICIAL SUBSTRATE PERIPHYTON DATA FROM GRANT CREEK, FALL, 1975

|      | Week 1                                   |                        |                        | Week 2                                   |                        |                        | Week 3                                   |                        |                        |
|------|--|------------------------|------------------------|--|------------------------|------------------------|--|------------------------|------------------------|
|      | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity |
| GC-1 | 1.5                                      | 14                     | 1.68                   | 6.6                                      | 28.3                   | 2.32                   | 22.8                                     | 43                     | 3.25                   |
| GC-2 | 1.2                                      | 14                     | 2.12                   | 4.4                                      | 36.3                   | 3.12                   | 11.6                                     | 34                     | 2.80                   |
| GC-3 | 1.7                                      | 9                      | 1.63                   | 1.6                                      | 16.7                   | 1.74                   | 1.0                                      | 10                     | 2.05                   |
| GC-4 | 4.7                                      | 20                     | 2.47                   | 6.7                                      | 21.3                   | 2.45                   | 3.6                                      | 19                     | 2.49                   |
| GC-5 | 8.6                                      | 17                     | 2.44                   | 11.3                                     | 24.7                   | 2.68                   | 7.6                                      | 20                     | 2.33                   |

The ANOVA performed on the periphyton from artificial substrates showed significant differences in algal communities in all parameters measured (Table 17). Station CC-3 consistently had the lowest means, significantly lower than both upstream stations GC-1 and GC-2 for numbers of species and diversity indices. The numbers of individuals at both GC-4 and GC-5 were higher than at any station above the pink water outfall yet the mean numbers of species at those stations were lower. These differences are attributable to shifts in diatom assemblages. The numbers of diatom species found there showed a significant reduction from the large variety found at GC-1 and GC-2, but showed a notable increase over those found at GC-3. The mean diversity index at GC-4 and GC-5 also showed a significant increase in comparison to GC-3.

TABLE 17. ANOVA OF ARTIFICIAL SUBSTRATE DATA  
FROM GRANT CREEK, FALL, 1975

| Station                      | Number of Individuals $\times 10^4$ | Station | Number of Species | Station | $\bar{H}$ |
|------------------------------|-------------------------------------|---------|-------------------|---------|-----------|
| GC-3                         | 1.6                                 | GC-3    | 16.7              | GC-3    | 1.74      |
| GC-2                         | 4.4                                 | GC-4    | 21.3              | GC-1    | 2.32      |
| GC-1                         | 6.6                                 | GC-5    | 24.7              | GC-4    | 2.45      |
| GC-4                         | 6.7                                 | GC-1    | 28.3              | GC-5    | 2.68      |
| GC-5                         | 11.3                                | GC-2    | 36.3              | GC-2    | 3.12      |
| Significance level of F-test | .99                                 |         | .99               |         | >.999     |

These data suggest a greater impact to the periphyton community from the filtration plant effluent (GC-3) than from the pink water effluent (GC-4). GC-4 and GC-5 may well be areas of impact from the pink water but may also be recovery zones from the effects of the filtration plant.

Biomass, Chlorophyll a and Autotrophic Index. Table 18 summarizes biomass and chlorophyll a data from Grant Creek during the fall survey. Stations GC-4 and GC-5 yielded the greatest biomass. In an undisturbed condition, large amounts of chlorophyll a would also be expected. However, pigment values are comparable to upstream stations which yielded considerably less biomass. In the case of GC-4 and GC-5 large amounts of grey-white matter coated with a red-brown slime covered the diatometer slides. This material was not autotrophic; it may have been bacteria or fungi or possibly non-organic material. The substance was not in evidence at GC-3, yet the chlorophyll a values were low here also. Non-autotrophic biomass at the station may have resulted from the non-chlorophyll containing gelatinous sheaths often associated with blue-gree algae, particularly abundant at this station. An acceptable proportion of biomass and chlorophyll a was found at GC-1 and GC-2 producing autotrophic indices less than 100. [Values in excess of 100 are considered indicative of organic pollution (Weber and McFarland, 1969)].

TABLE 18. BIOMASS, CHLOROPHYLL a, AND AUTOTROPHIC INDICES FOR GRANT CREEK, FALL, 1975

|      | Biomass<br>mg/m <sup>2</sup> | Chlorophyll <u>a</u><br>mg/m <sup>2</sup> | Autotrophic Index<br>Biomass/Chl <u>a</u> |
|------|------------------------------|---|---|
| GC-1 | 1209.9                       | 16.83                                     | 71.9                                      |
| GC-2 | 1399.3                       | 15.28                                     | 91.6                                      |
| GC-3 | 1003.2                       | 3.13                                      | 320.4                                     |
| GC-4 | 4512.2                       | 17.38                                     | 259.6                                     |
| GC-5 | 8546.5                       | 23.14                                     | 369.4                                     |

Colonization. A consistent pattern of colonization of the artificial substrates can be seen at GC-1 and GC-2 during the fall survey (Table 17). GC-1 showed its most diverse community at 3 weeks, GC-2 at 2 weeks. Numbers of individuals increased approximately four times between weeks 1 and 2, approximately three times between weeks 2 and 3. GC-3 showed its most diverse community at 3 weeks, however, the highest numbers of individuals and species occurred during the second week. Relatively similar patterns of colonization occurred at GC-4 and GC-5 as was seen at GC-3. A greater decrease in the number of individuals occurred between weeks 2 and 3. At these two stations the decrease can be attributed to sluffing of organisms, a phenomenon enhanced by accumulation of a grey-white slimy matter on the diatomometer slides.

In the case of GC-1 and GC-2, new organisms were still colonizing the slides at 3 weeks. At GC-3, GC-4, and GC-5, third-week changes involved primarily loss of populations of organisms dominant during the second week, and increased populations of a few organisms in a situation of reduced competition.

Figure 8 presents dendograms of cluster analyses on periphyton data from all five Grant Creek stations for natural (A) and artificial (B) substrates.

In both dendograms, the two downstream stations, GC-4 and GC-5, and the two upstream stations, GC-1 and GC-2, formed a cluster by themselves. Beyond these initial steps the dendograms differed.

The dendrogram for natural substrate data demonstrated a major difference in species assemblages between the groups of stations upstream (GC-1 and GC-2) and downstream (GC-3, GC-4, GC-5) of the water treatment plant outfall since the merge between these groups is the lowest on the tree. A lesser degree of difference was shown between stations upstream (GC-3) and downstream (GC-4, GC-5) of the TNT outfall. This dendrogram indicates an impact on the natural substrate species assemblages from the water treatment plant outfall at GC-3. However, it does not determine whether or not this impact was actually greater than the impact at GC-4 and GC-5 caused by the TNT outfall.

The dendrogram for data from artificial substrates shows that the two upstream stations merge with the two downstream stations at a low level of similarity (.10) prior to the final merger of GC-3 at .08. This dissimilarity of GC-3 demonstrated the impact from the water treatment plant effluent on periphyton species assemblages. However, there was almost as great a difference between GC-1 and GC-2 and GC-4 and GC-5; the two downstream stations may be areas affected by the TNT outfall, or they may be areas showing a small recovery from the impact of the water treatment outfall. These conclusions agree with the overall results of the ANOVA performed on the artificial community structure variables.

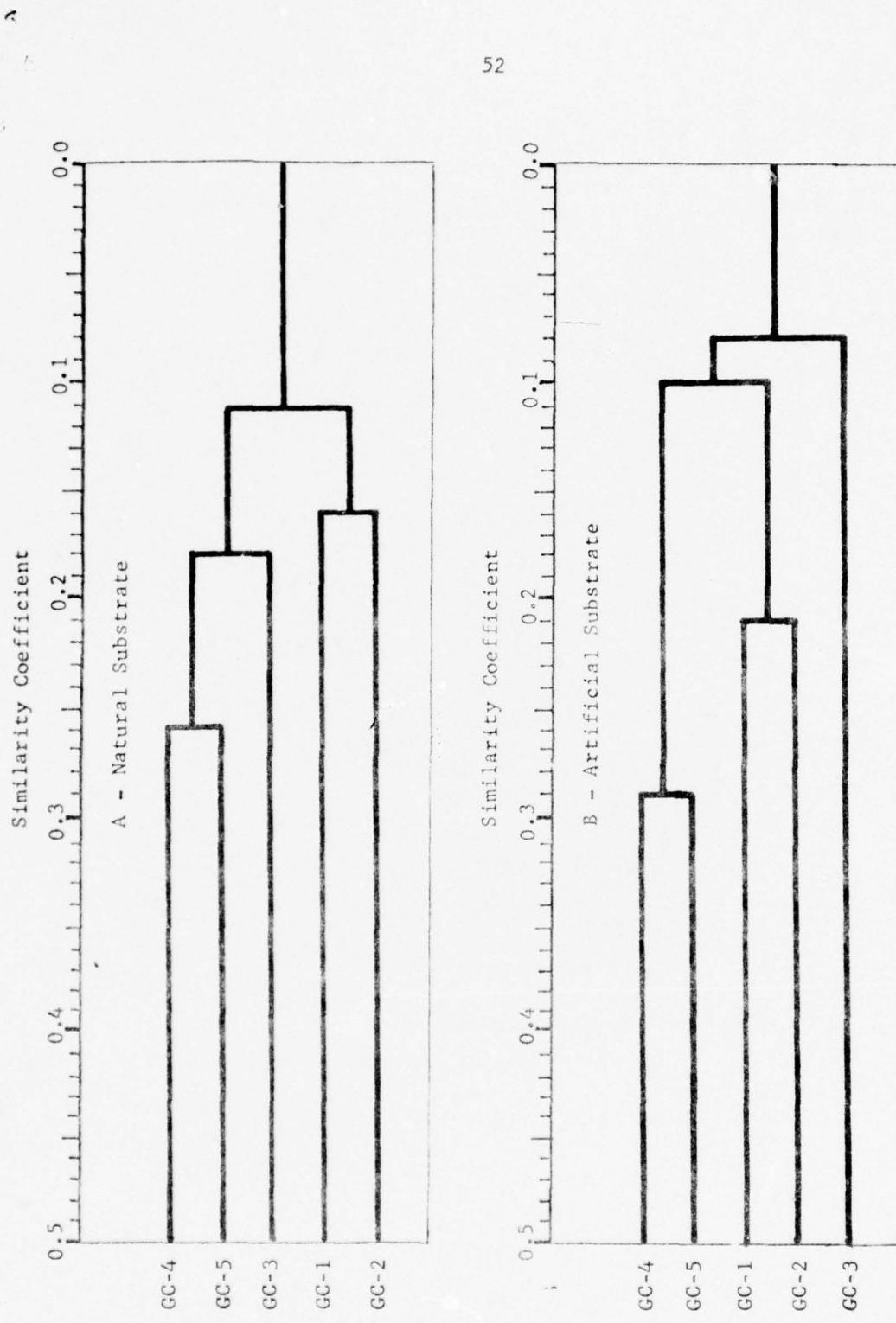


FIGURE 8. DENDOGRAM OF GRANT CREEK PERIPHYTON DATA COLLECTED FROM NATURAL (A) AND ARTIFICIAL (B) SUBSTRATES, FALL, 1975

Grant Creek Benthic Macroinvertebrates (Spring)

Species Composition. Benthic macroinvertebrate collections made during the spring survey indicated the presence of more species downstream of the pink water discharge (GC-4 and GC-5) than upstream (GC-1 and GC-2) on both natural and artificial substrates. The fewest number of species were found at GC-3 indicating a residual effect from the water treatment plant effluent. Due to the unique conditions existing at GC-3, this station was not included with either upstream or downstream stations in any calculations or comparisions.

Although larger numbers of species were found downstream, certain population shifts did occur. The black fly larva, Simulium sp., was found to be a dominant organism in upstream collections from both natural and artificial substrates but was never collected below the pink water outfall. Habitat variations may partially explain the lack of this species. Bedrock substrates in this channelized portion of Grant Creek do not provide suitable conditions for Simulium colonization. Another dominant invertebrate upstream, Asellus sp. (Isopoda), had greatly reduced numbers at GC-4 and GC-5. The dominant organism on artificial substrates at these downstream stations was a midge, Cricotopus sp. No clear dominance of any invertebrates was observed in the natural substrates. Clearly, dominance and species composition are different between the stations above and below the addition of "pink water".

A complete species checklist of macroinvertebrate organisms collected from the natural substrates and with artificial substrates from Grant Creek in the spring of 1975 appear in Appendix D, Tables 6 and 12, respectively.

Community Structure. A variety of parameters describing community structure including standing crop, number of species, species diversity ( $H'$ ) and colonization rate are summarized in Table 19. Individual sample data are presented in Appendix D, Tables 1 through 11. Colonization rates were calculated from numbers of species and individuals collected with artificial substrates at the three different incubation periods presented in Table 19. These rates will be discussed later in this section.

A review of natural substrate data presented in Table 19 indicated a lower standing crop of invertebrates inhabiting areas below the "pink water" discharge. However, data generated from artificial substrate samplers are inconsistent for the 3 weeks of incubation. In order to identify significant differences among the sampling stations for the various parameters, an analysis of variance and a Duncan's mean separation test were performed on the data. Table 20 presents the results of these analyses.

About half of the analyses of variance performed on benthic macro-invertebrate data collected in the spring from Grant Creek, showed significant differences in community structure variables among the three groups of sampling stations.

The natural substrate data showed upstream stations to have significantly higher numbers of individuals (standing crop) than downstream sites.

The artificial substrate samples showed no significant differences among any of the community structure variables during week three. However, GC-3 had the lowest mean diversity index during this week, due to the large numbers of Asellus sp. At GC-3, this species was at a peak during week 3, but declined gradually through the following two weeks.

During sampling week 4, all community structure variables showed significant differences; GC-4 and GC-5 had the highest mean numbers of individuals but GC-5 as well as GC-4 had the lowest mean diversity indices. This was caused by the large peak in numbers of Cricotopus sp. which occurred at both GC-4 and GC-5 during this week.

Samples collected during sampling week 5 showed significant differences in numbers of individuals and diversity indices. During this week, the mean separation test placed GC-1 in a group by itself (Samplers at GC-2 were lost before the fifth week), having the highest mean number of individuals, but also the lowest mean diversity index. Both of these extremes were caused by large numbers of Asellus sp. at GC-1. This species dominated the invertebrate community at GC-1 during all three sampling weeks.

Figure 9 presents dendograms of cluster analyses performed on macro-invertebrate data from all five Grant Creek stations, separately for natural (a) and artificial (b) substrate samples.

TABLE 19. SUMMARY OF ANALYSES OF THE BENTHIC MACROINVERTEBRATE DATA FROM NATURAL AND ARTIFICIAL SUBSTRATE SAMPLES FROM GRANT CREEK, SPRING, 1975

TABLE 20. ANOVA ON BENTHIC MACROINVERTEBRATE  
DATA FROM GRANT CREEK, SPRING, 1975

|                                 | Number of<br>Site Indiv. |       | Number of<br>Site Indiv. |      | Site | H     |
|---------------------------------|--------------------------|-------|--------------------------|------|------|-------|
| <u>Natural Substrates</u>       |                          |       |                          |      |      |       |
|                                 | GC-4                     | 10.2  | GC-3                     | 4.2  | GC-3 | 1.08  |
|                                 | GC-5                     | 10.4  | GC-4                     | 4.8  | GC-1 | 1.12  |
|                                 | GC-3                     | 20.4  | GC-5                     | 6.0  | GC-4 | 1.24  |
|                                 | GC-1                     | 37.4  | GC-1                     | 6.2  | GC-2 | 1.28  |
|                                 | GC-2                     | 60.8  | GC-2                     | 6.2  | GC-5 | 1.49  |
| Significance Level<br>of F-test |                          | .99   |                          | —    |      | —     |
| <u>Artificial Substrates</u>    |                          |       |                          |      |      |       |
| WEEK 3                          |                          |       |                          |      |      |       |
|                                 | GC-4                     | 22.5  | GC-5                     | 7.0  | GC-3 | 0.86  |
|                                 | GC-1                     | 48.5  | GC-4                     | 8.0  | GC-5 | 1.27  |
|                                 | GC-3                     | 67.0  | GC-3                     | 8.5  | GC-2 | 1.43  |
|                                 | GC-5                     | 70.0  | GC-2                     | 9.0  | GC-1 | 1.47  |
|                                 | GC-2                     | 124.0 | GC-1                     | 9.5  | GC-4 | 1.89  |
| Signif. Level of F-test         |                          | —     |                          | —    |      | —     |
| WEEK 4                          |                          |       |                          |      |      |       |
|                                 | GC-4                     | 91.2  | GC-4                     | 6.2  | GC-5 | 0.73  |
|                                 | GC-2                     | 100.8 | GC-2                     | 7.6  | GC-4 | 0.90  |
|                                 | GC-1                     | 105.6 | GC-1                     | 9.6  | GC-2 | 0.98  |
|                                 | GC-3                     | 121.2 | GC-3                     | 10.2 | GC-1 | 1.33  |
|                                 | GC-5                     | 228.6 | GC-5                     | 10.2 | GC-3 | 1.44  |
| Signif. Level of F-test         |                          | >.999 |                          | >.95 |      | >.999 |
| WEEK 5                          |                          |       |                          |      |      |       |
|                                 | GC-4                     | 13.0  | GC-4                     | 6.5  | GC-1 | 1.12  |
|                                 | GC-5                     | 21.5  | GC-5                     | 7.0  | GC-3 | 1.60  |
|                                 | GC-3                     | 32.0  | GC-1                     | 8.5  | GC-4 | 1.60  |
|                                 | GC-1                     | 55.0  | GC-3                     | 10.0 | GC-5 | 1.67  |
| Signif. Level of F-test         |                          | .99   |                          | —    |      | .95   |

— no significant difference

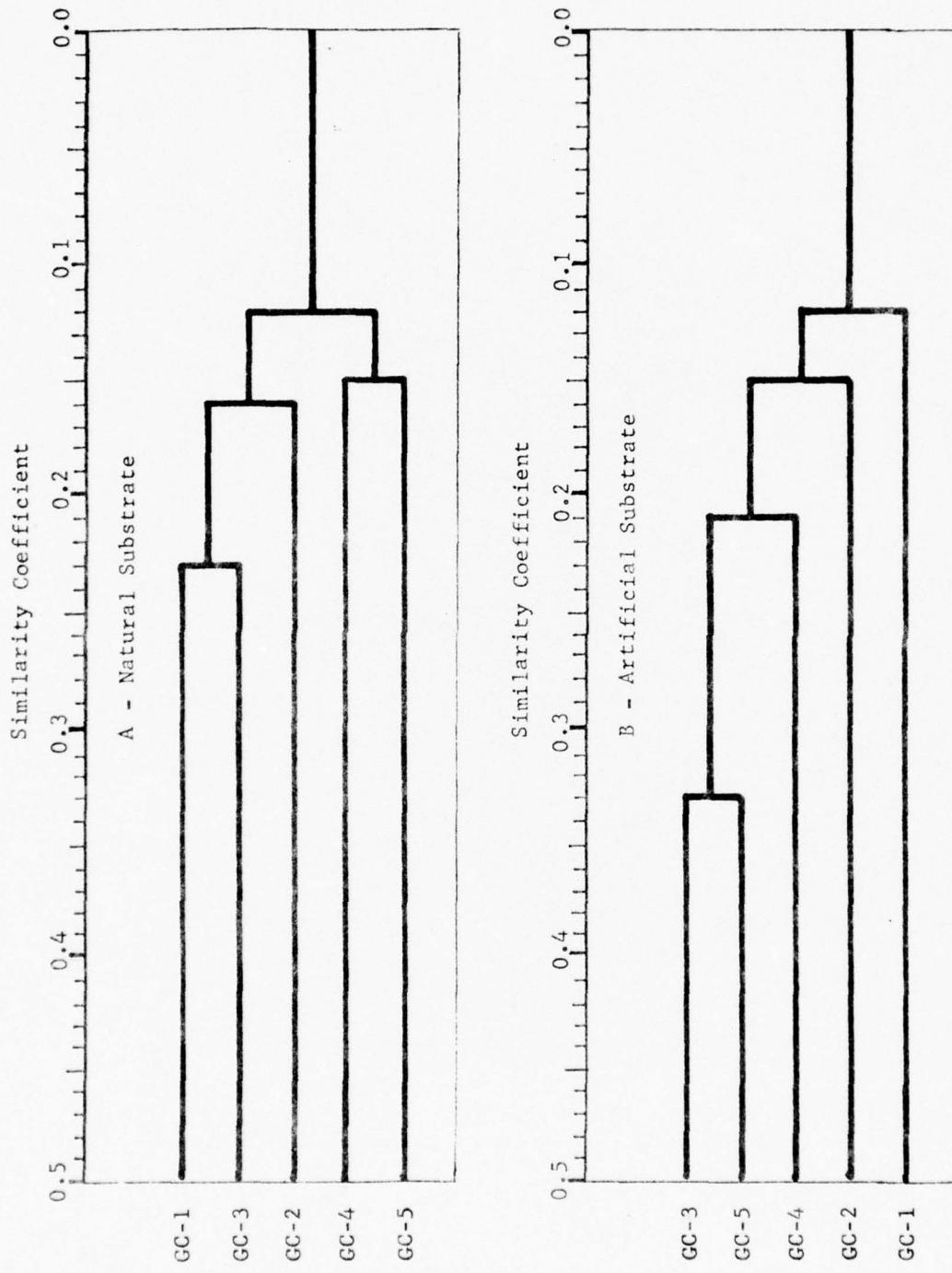


FIGURE 9. DENDOGRAM OF GRANT CREEK BENTHIC MACROINVERTEBRATE DATA COLLECTED FROM NATURAL (A) AND ARTIFICIAL (B) SUBSTRATES, SPRING, 1975

In the dendrogram for natural substrate samples, the three main steps which took place were (1) the three upstream stations GC-1, GC-2, and GC-3 merge together at a level of 0.16; (2) the two downstream stations, GC-4 and GC-5, merge together at a level of 0.15; and (3) the cluster for the upstream stations merged with the cluster for the downstream stations at a level of 0.12.

In the dendrogram for artificial substrate samples, the main steps which took place were (1) the three stations downstream of the water treatment plant outfall, GC-3, GC-4, and GC-5, merged together at a level of 0.21; and (2) GC-2 and GC-1 merged one at a time with the first cluster, at levels of 0.15 and 0.12, respectively.

The difficulty in interpreting these results lies in the relationship of GC-3 to the other Grant Creek stations. GC-3 pairs closely with the upstream station GC-1 in the natural substrate dendrogram, but pairs closely with the downstream station GC-5 in the artificial substrate dendrogram. This situation may be caused by scouring of the artificial substrate samples at GC-3 by the flocculant material contained in the water treatment plant effluent. The high flows experienced at this time may have kept this abrasive material in suspension above the natural substrates reducing the impact on the organisms inhabiting these areas.

If GC-3 is omitted from consideration, both dendograms show a subtle difference between the species assemblages found at the upstream stations (GC-1 and GC-2) and those found at the downstream stations (GC-4 and GC-5). However, this difference is not very clearly demonstrated, because there are still sizeable differences between assemblages found at GC-1 and GC-2, and also between assemblages found at GC-4 and GC-5. Therefore, the impact of the TNT effluent upon the downstream stations is minor during the spring sampling period.

Colonization Rate. The rate of colonization by individuals and species was calculated from the three sets of artificial substrate data and is presented graphically in Figure 10. Dotted lines represent the mean values for study stations and solid lines portray control stations over the 3-week incubation period. During this time it appears the control stations had already reached peak populations of individuals and a stable number of species. However, below the "pink water" discharge, the benthic invertebrate community

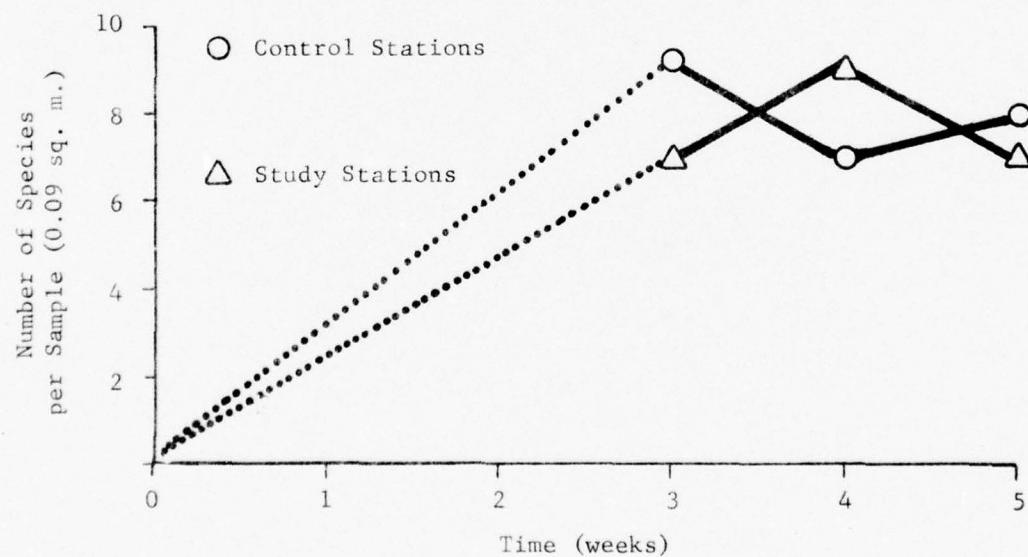
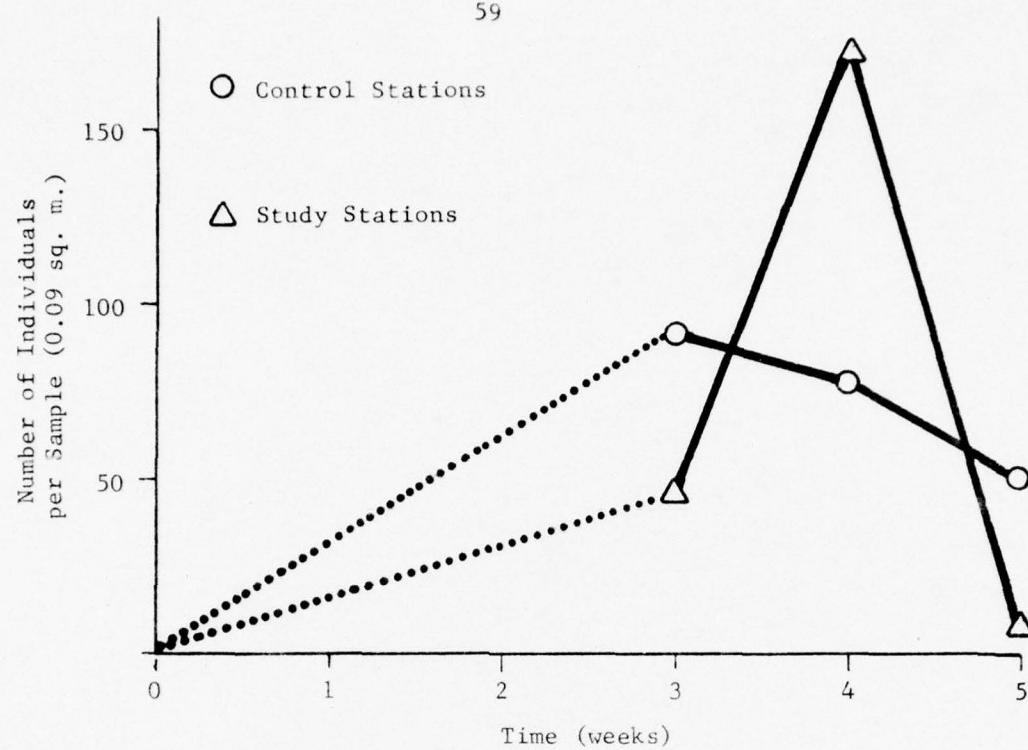


FIGURE 10. BENTHIC MACROINVERTEBRATE COLONIZATION RATE OF ARTIFICIAL SUBSTRATES IN GRANT CREEK, SPRING, 1975

was dominated by large numbers of the midge, Cricotopus sp. which emerged between the fourth and fifth weeks of incubation. This emergence caused the dramatic reduction in standing crop observed in Figure 10. Upstream emergence of this species occurred at approximately the same time, but many fewer individuals of this species were present in the upstream invertebrate community. Little difference was observed in the rate of increase in number of species. It appeared that both areas reached peak numbers of species before the third week and had leveled off.

#### Grant Creek Benthic Macroinvertebrates (Fall)

Flow conditions were low in Grant Creek during the fall of 1975. Sampling with a Surber sampler at GC-1 was impossible as there was no flow in this segment of Grant Creek. Artificial substrate samplers were located in a pool-run area and were submerged in water for the entire incubation period. This low flow period allowed the flocculant material in the filtration plant effluent to settle out along the shorelines of Grant Creek as far downstream as GC-5. The material was often 8 to 10 cm deep or deeper along the stream edge and completely covered the bottom substrates. This covering created an inhospitable environment for benthic macroinvertebrate colonization at GC-3 and made the determination of munitions waste effects on this group of organisms difficult.

Species Composition. The species present at the Grant Creek stations in the fall were quite similar to those found in the spring. Diptera larvae comprised over 50 percent of the species collected from each site. A summary of the total number of species collected on natural and artificial substrates from upstream (GC-1 and GC-2), GC-3, and downstream (GC-4 and GC-5) is presented in Table 21.

TABLE 21. NUMBERS OF BENTHIC MACROINVERTEBRATE SPECIES COLLECTED FROM GRANT CREEK, FALL, 1975

|            | Natural | Artificial |
|------------|---------|------------|
| Upstream   | 15      | 37         |
| GC-3       | 4       | 5          |
| Downstream | 15      | 29         |

The numbers of species found on natural and artificial substrates at GC-3 were only 4 and 5, respectively. This lower number of species is indicative of an environmental stress exerted by the water treatment plant effluent on the invertebrates at GC-3.

Recovery is observed at the further downstream stations but several population shifts are observed. The Isopod Asellus sp. (dominant at upstream stations) is again found as in the spring, in much fewer numbers below the "pink water" discharge. The black fly larva Simulium sp. (also numerous upstream) was never collected below the outfall. Another invertebrate, a mayfly, Baetis sp., which was found at GC-2 was not found at GC-3, GC-4, or GC-5.

It is difficult to ascertain the cause of these population shifts due to the tremendous impact observed at station GC-3. However, the recovery of numbers of species at the downstream sites in the fall, coupled with the fact that two of these species were similarly affected in the spring, would indicate that it is indeed the "pink water" discharge causing these population shifts. Species check lists for natural and artificial substrate samples appear in Appendix D, Tables 18 and 24. Complete lists of species and numbers in each individual sample from Grant Creek are presented in Appendix D, Tables 13 through 23.

Community Structure. A review of the parameters outlined in Table 22, which presents invertebrate community structure data collected from natural and artificial substrates from Grant Creek, further demonstrates the environmental perturbation created by the flocculant material contained in the filtration plant effluent. All parameters calculated, including number of individuals per sample, number of species, and species diversity were much lower at GC-3 than at the control stations. Some recovery from this stress occurred at GC-4 and GC-5 for number of species and species diversity, but the number of individuals was still much lower than expected.

Analyses of variance were performed on these Grant Creek sample data as in the spring sampling, to test for differences between the five sampling stations. The results of these analyses and Duncan's mean separation tests are presented in Table 23.

TABLE 22. SUMMARY OF ANALYSES OF THE BENTHIC-MACROINVERTEBRATE DATA FROM NATURAL AND ARTIFICIAL SUBSTRATE SAMPLES FROM GRANT CREEK, FALL, 1975.

| Natural Substrate | Artificial Substrate |        |        |                            |                        | Mean Species Diversity |
|-------------------|----------------------|--------|--------|----------------------------|------------------------|------------------------|
|                   | Week 2               | Week 3 | Week 4 | Mean Number of Individuals | Mean Number of Species |                        |
| GC-1              | --                   | --     | --     | 63.0                       | 8.5                    | 1.69                   |
| GC-2              | 111.2                | 6.6    | 1.02   | 51.5                       | 10.0                   | 1.94                   |
| GC-3              | 2.0                  | 1.2    | 0.39   | 1.0                        | 0.5                    | 0.00                   |
| GC-4              | 6.8                  | 4.0    | 1.23   | 21.0                       | 9.0                    | 1.95                   |
| GC-5              | 6.2                  | 3.2    | 0.90   | 5.0                        | 4.0                    | 1.21                   |
|                   |                      |        |        |                            |                        | 10.0                   |
|                   |                      |        |        |                            |                        | 3.27                   |
|                   |                      |        |        |                            |                        | 2.67                   |
|                   |                      |        |        |                            |                        | 0.81                   |
|                   |                      |        |        |                            |                        | 1.71                   |
|                   |                      |        |        |                            |                        | 5.5                    |
|                   |                      |        |        |                            |                        | 4.5                    |
|                   |                      |        |        |                            |                        | 1.43                   |

TABLE 23. ANOVA ON BENTHIC MACROINVERTEBRATE  
DATA FROM GRANT CREEK, FALL, 1975

|  | Number of<br>Site Indiv. |      | Number of<br>Site Indiv. |       | Site H |
|--|--------------------------|------|--------------------------|-------|--------|
| <u>Natural Substrates</u> <sup>(1)</sup> |                          |      |                          |       |        |
| GC-3                                     | 2.0                      | GC-3 | 1.2                      | GC-3  | 0.39   |
| GC-5                                     | 6.2                      | GC-5 | 3.2                      | GC-5  | 0.09   |
| GC-4                                     | 6.8                      | GC-4 | 4.0                      | GC-2  | 1.02   |
| GC-2                                     | 111.2                    | GC-2 | 6.6                      | GC-4  | 1.23   |
| Signif. Level of F-test                  | .999                     |      | .995                     |       | .90    |
| <u>Artificial Substrates</u>             |                          |      |                          |       |        |
| WEEK 2                                   |                          |      |                          |       |        |
| GC-3                                     | 1.0                      | GC-3 | 0.5                      | GC-3  | 0.00   |
| GC-5                                     | 5.0                      | GC-5 | 4.0                      | GC-5  | 1.21   |
| GC-4                                     | 21.0                     | GC-1 | 8.5                      | GC-1  | 1.69   |
| GC-2                                     | 51.5                     | GC-4 | 9.0                      | GC-2  | 1.94   |
| GC-1                                     | 63.0                     | GC-2 | 10.0                     | GC-10 | 1.95   |
| Signif. Level of F-test                  | .99                      |      | .98                      |       | .98    |
| WEEK 3                                   |                          |      |                          |       |        |
| GC-3                                     | 0.7                      | GC-3 | 0.7                      | GC-3  | 0.00   |
| GC-4                                     | 3.7                      | GC-4 | 2.7                      | GC-4  | 0.81   |
| GC-5                                     | 8.3                      | GC-5 | 6.31                     | GC-1  | 1.69   |
| GC-1                                     | 44.3                     | GC-1 | 10.0                     | GC-5  | 1.79   |
| GC-2                                     | 78.0                     | GC-2 | 14.7                     | GC-2  | 2.17   |
| Signif. Level of F-test                  | .999                     |      | .999                     |       | .999   |
| WEEK 4                                   |                          |      |                          |       |        |
| GC-3                                     | 1.5                      | GC-3 | 1.0                      | GC-3  | 0.32   |
| GC-5                                     | 5.5                      | GC-5 | 4.5                      | GC-1  | 1.39   |
| GC-4                                     | 10.0                     | GC-1 | 7.0                      | GC-5  | 1.42   |
| GC-1                                     | 32.5                     | GC-4 | 7.5                      | GC-4  | 1.95   |
| GC-2                                     | 62.0                     | GC-2 | 11.0                     | GC-2  | 2.02   |
| Signif. Level of F-test                  | .999                     |      | .999                     |       | .99    |

(1) Surber samples were not collected at GC-1.

The ANOVA performed on natural substrate data showed that at GC-3 the means for all three community structure variables were (1) lower than those at all other Grant Creek stations, and (2) significantly lower than those at the upstream station GC-2. (No natural substrate sample could be taken at GC-1 due to low flow.) The Duncan's mean separation showed the mean number of individuals at GC-2 to be significantly higher than, and on the order of 20 times as large as, the mean numbers of individuals for GC-3, GC-4, and GC-5. This large standing crop at GC-2 consisted predominantly of Asellus sp. At GC-4 and GC-5 no single species showed dominance, and each of the relatively few species sampled were found in low densities. At GC-3, both density and diversity were extremely low.

The ANOVA performed on artificial substrate data showed that at GC-3 the means for all three community structure variables, during all three sampling weeks, were (1) lower than those at all other Grant Creek stations, and (2) significantly lower than those at the upstream stations GC-1 and GC-2. Since the same results were observed for the natural substrate data, it can be concluded that the area around GC-3 was inhospitable to benthic macroinvertebrate communities during the fall sampling season. Undoubtedly, this area was adversely affected by the flocculant materials that precipitated from the filtration plant effluent just upstream of GC-3. During the fall these materials accumulated along the stream shorelines. Little or no effect was observed at GC-3 during the spring, presumably because the higher water levels and faster stream currents flushed the stream of this material.

The ANOVAs on artificial substrate data also showed that for all three sampling weeks, (1) mean numbers of individuals at GC-1 and GC-2 were significantly higher than at GC-4 and GC-5, (2) numbers of species at GC-1 and GC-2 were significantly higher than at GC-5, (3) mean diversity indices at GC-1 and GC-2 were similar to those at GC-4 or GC-5, or both. Relative to upstream control stations, GC-4 and GC-5 may be zones affected by the TNT outfall, and/or zones of partial recovery from the filtration plant effluent. All three variables had higher means at GC-5 than at GC-4 during week 4, but during weeks 3 and 5 the reverse was true. Thus, there was no clear indication that benthic communities at GC-5 exhibited a greater degree of recovery than those at GC-4.

The benthic macroinvertebrate communities found at GC-1 and GC-2 during the incubation period were characterized by a large variety of midge species. Chironomus (chironomus) sp. and Tanytarsus sp. were especially prevalent at both GC-1 and GC-2 during all sampling weeks; however, no single midge species was so dominant as to cause low diversities. GC-4 and GC-5 each had a large variety of macroinvertebrate species though none were found in large numbers. At GC-3 both densities and diversity were extremely low.

Figure 11 presents dendograms of cluster analyses performed on macroinvertebrate data from Grant Creek stations, separately for natural (A) and artificial (B) substrate samples. The natural substrate dendrogram uses only four sampling stations instead of five, because no samples were collected at GC-1 in the fall due to low flow conditions.

In the dendrogram for natural substrate samples the steps which took place were (1) the downstream stations GC-4 and GC-5 merged together at a level of 0.34; (2) GC-2 and GC-3 merged together at a level of 0.20; and (3) the two clusters from (1) and (2) merged together at a level of only 0.08.

Step (2) above may require some comments. So few species were present in the natural substrate community at GC-3 that it might appear to make this station very dissimilar to all others. However, all of the few species found there were also at GC-2, accounting for the merge that took place between GC-3 and GC-2.

The main conclusion that this cluster analysis suggests is that there is a moderate difference between the species assemblages found on natural substrates at the sampling stations above the TNT outfall (GC-2 and GC-3) and those found at the stations below the outfall (GC-4 and GC-5). Also, there is a lesser difference between species assemblages found above (GC-2) and below (GC-3) the water treatment plant outfall.

In the dendrogram for artificial substrate samples, the steps which took place were (1) the two downstream stations GC-4 and GC-5 merged at a level of 0.33; (2) the two upstream stations GC-1 and GC-2 merged at a level of 0.23;

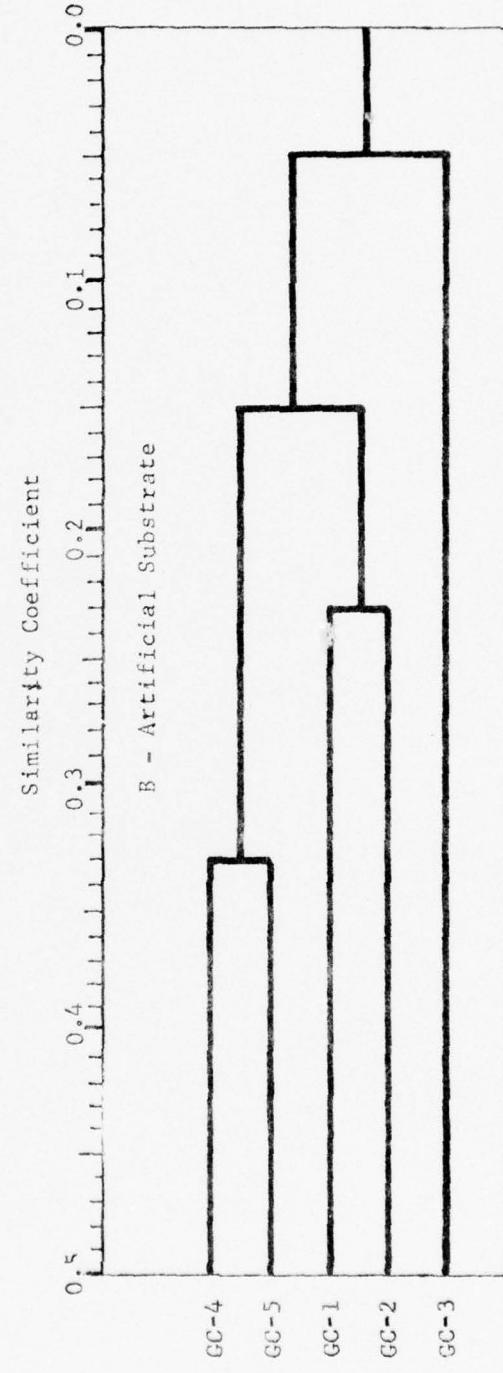
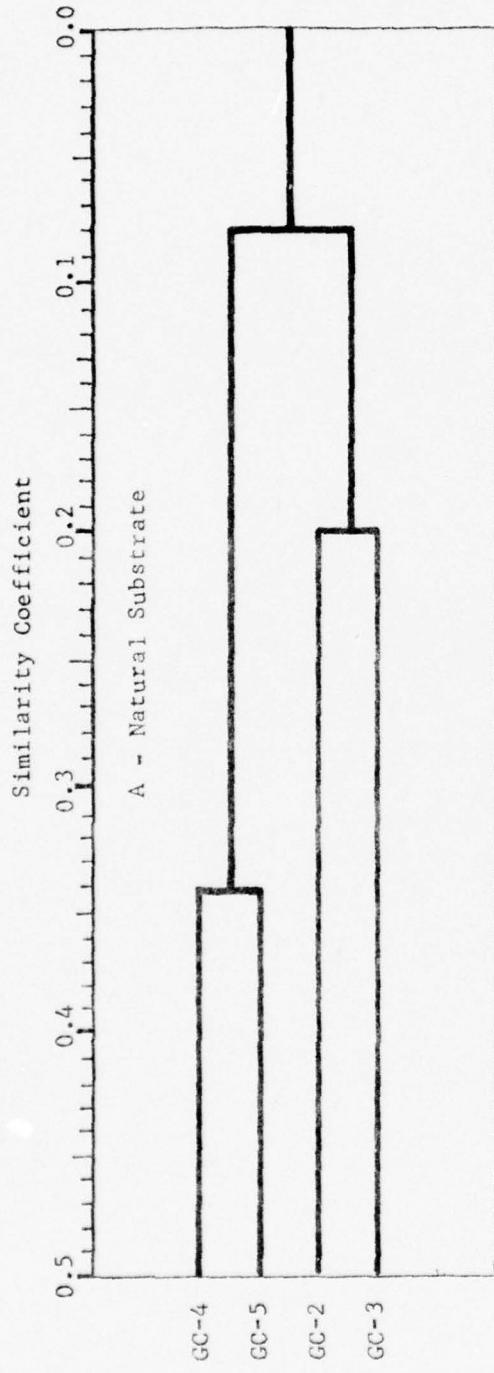


FIGURE 11. DENDOGRAM OF GRANT CREEK BENTHIC MACROINVERTEBRATE DATA COLLECTED FROM NATURAL (A) AND ARTIFICIAL (B) SUBSTRATES, FALL, 1975

(3) the two clusters from (1) and (2) joined at a level of 0.15; and (4) GC-3 joined the cluster for all the other stations at a level of only 0.05.

This cluster analysis suggested that there was a major difference between the species assemblages found on artificial substrates at GC-3 and those found at all other Grant Creek stations. There is also a lesser difference between the species assemblages found at the upstream (GC-1 and GC-2) and downstream (GC-4 and GC-5) stations.

This dendrogram therefore shows that the primary impact upon the Grant Creek species assemblages is due to the water treatment plant effluent, with the TNT effluent causing a secondary impact.

Doyle LakeWater Quality

Table 24 is a summary of the analysis from the spring and fall sampling effort at the Doyle Lake sites. The table also includes selected water quality criteria. The complete data sets utilized to compose this summary table appear in Appendix A, Tables 1 and 2. The sample sites are described in an annotated legend (see Table 1) and located in Figure 1.

The total dissolved solids concentrations (TDS) are regionally slightly high throughout the sampling stations and times. Calcium, magnesium, carbonates, sulfates and, to a lesser extent, chlorides are the major contributor to these slightly elevated TDS values. Spring and fall nutrient concentrations in the Doyle Lake system are also high. The mean nitrate concentration is high enough (75 mg/l, with a maximum value of 195) that it could be a hazard to livestock (National Science Foundation, 1973). Phosphate concentrations are also significantly elevated.

Sediment Chemistry

A summary of the sediment characteristics from the combined spring and fall data from Doyle Lake is presented in Table 25. Concentrations defining polluted sediments are also given for comparison.

The sediments are considered polluted with respect to the reported macronutrients nitrogen and phosphorus with mean values greater than 1000 and 900 mg/l, respectively. The sediments are also judged "lightly" polluted in regard to the chemical oxygen demand potential. The resuspension of these sediments results in significant oxygen reductions as evidenced by dissolved oxygen trends during the spring sampling period (DL-2, DL-3 and DL-4).

TABLE 24. WATER QUALITY DATA FROM DOYLE LAKE, SPRING AND FALL, 1975

| Selected Criteria                | Field Measurements |       |           |      |      |              | Laboratory Measurements (ppm) |          |         |         |                 |                 |                 |                 |                 |      |     |     | Number of Samples Considered |
|----------------------------------|--------------------|-------|-----------|------|------|--------------|-------------------------------|----------|---------|---------|-----------------|-----------------|-----------------|-----------------|-----------------|------|-----|-----|------------------------------|
|                                  | pH                 | Cond. | Temp., °C | D.O. | Alk. | Total Solids | Diss. Solids                  | CDOD (a) | TOK (b) | TKN (c) | NH <sub>4</sub> | NO <sub>2</sub> | NO <sub>3</sub> | PO <sub>4</sub> | SO <sub>4</sub> | C1   | Lab |     |                              |
|                                  | 6-9 <900           | -     | >5        | -    | -    | <80          | <500                          | <40      | <12     | <1.0    | <0.02           | -               | <1.5            | <0.1            | <250            | <250 |     |     |                              |
| <u>Spring</u>                    |                    |       |           |      |      |              |                               |          |         |         |                 |                 |                 |                 |                 |      |     |     |                              |
| Discharge to Doyle Lake (DL-0,1) | Mean               | 7.4   | 1272      | 20.5 | 6.7  | 250          | 204                           | 773      | 18      | 6.7     | <0.18           | 0.13            | 13.3            | 0.08            | 192             | 122  | 10  |     |                              |
|                                  | S                  | 0.29  | 173       | 1.91 | 0.94 | 12           | 26                            | 72       | 6.7     | 2.3     | <0.39           | 0.23            | 22.5            | 0.03            | 27              | 26   |     |     |                              |
|                                  | Max                | 7.9   | 1516      | 23.8 | 7.9  | 264          | 234                           | 874      | 35      | 11      | 1.3             | 0.75            | 76.0            | 0.14            | 229             | 168  |     |     |                              |
|                                  | Min                | 6.9   | 1092      | 18.1 | 5.3  | 231          | 172                           | 676      | 10      | 5       | 0.5             | <0.05           | 3.0             | 0.04            | 162             | 74   |     |     |                              |
| Doyle Lake (DL-2,3)              | Mean               | 7.9   | 596       | 23.4 | 6.5  | 191          | 249                           | 385      | 32      | 7.7     | 1.5             | 0.33            | 75              | 0.06            | 59              | 19.5 | 6   |     |                              |
|                                  | S                  | 0.17  | 73        | 3.0  | 1.6  | 19           | 21                            | 39       | 10      | 1.6     | 0.3             | 0.09            | 86              | 0.03            | 5.4             | 1.9  |     |     |                              |
|                                  | Max                | 7.6   | 719       | 28.3 | 8.4  | 218          | 275                           | 124      | 40      | 10      | 1.9             | 0.05            | 195             | 0.11            | 66              | 22   |     |     |                              |
|                                  | Min                | 6.1   | 513       | 20.2 | 2.7  | 121          | 222                           | 18       | 370     | 23      | 6               | <0.05           | 0.24            | 17              | 0.02            | 52   | 17  |     |                              |
| Doyle Lake Outflow (DL-4)        | High               | 8.5   | 598       | 28.6 | 7.7  | 220          | 249                           | 86       | 378     | 10      | 2.2             | 0.06            | 0.34            | 17.0            | 0.11            | 71   | 21  |     |                              |
|                                  | Low                | 7.7   | 528       | 20.3 | 3.8  | 185          | 218                           | 36       | 362     | 12      | 8               | <0.05           | 0.23            | 1.0             | 0.03            | 50   | 18  |     |                              |
| <u>Fall</u>                      |                    |       |           |      |      |              |                               |          |         |         |                 |                 |                 |                 |                 |      |     |     |                              |
| Discharge to Doyle Lake (DL-0,1) | Mean               | 7.8   | 927       | 17.3 | 6.8  | 268          | 263                           | 55       | 655     | 12      | 6               | 0.45            | 0.13            | 0.06            | 4.0             | 0.37 | 121 | 99  |                              |
|                                  | S                  | 0.4   | 355       | 2.2  | 1.5  | 90           | 163                           | 55       | 287     | 9.8     | 3.5             | 0.3             | 0.10            | 0.04            | 2.6             | 103  | 69  |     |                              |
|                                  | Max                | 8.2   | 1650      | 21.0 | 8.6  | 343          | 389                           | 126      | 1024    | 24      | 9               | 0.8             | 0.25            | 0.11            | 7.0             | 0.65 | 270 | 199 |                              |
|                                  | Min                | 6.9   | 280       | 14.5 | 4.5  | 149          | 115                           | 16       | 324     | <4      | 3               | <0.2            | <0.05           | <0.02           | 1.0             | 0.15 | 32  | 41  |                              |
| Doyle Lake (DL-2,3)              | Mean               | 7.8   | 946       | 18.4 | 7.5  | 253          | 214                           | 47       | 633     | 4.3     | 15              | 1.3             | 0.08            | 0.17            | 131             | 95   | 4   |     |                              |
|                                  | S                  | 0.23  | 70        | 1.3  | 2.0  | 1            | 12                            | 17       | 7.4     | 1.4     | 0.34            | 0.02            | 0.07            | 1               |                 |      |     |     |                              |
|                                  | Max                | 8.1   | 1010      | 20.8 | 10.2 | 254          | 216                           | 58       | 646     | 52      | 16              | 1.5             | 0.11            | <0.02           | <1.0            | 0.27 | 132 | 96  |                              |
|                                  | Min                | 7.4   | 827       | 17.2 | 5.2  | 252          | 211                           | 32       | 608     | 34      | 13              | 0.8             | 0.05            | 0.05            | 0.11            | 12.9 | 94  |     |                              |
| Doyle Lake Outflow (DL-4)        | High               | 8.4   | 900       | 23.0 | 20.6 | 359          | 359                           | 246      | 570     | 30      | 16              | 2.5             | 0.18            | <0.02           | <1.0            | 0.08 | 77  | 83  |                              |
|                                  | Low                | 8.1   | 350       | 15.7 | 4.3  | 155          | 176                           | 54       | 254     | 10      | 8               | 1.1             | 0.11            | 0.06            | 0.06            | 31   | 20  |     |                              |

(a) Chemical Oxygen Demand

(b) Total Organic Carbon

(c) Total Kjeldahl Nitrogen

TABLE 25. SUMMARY OF SEDIMENT CHARACTERISTICS FOR COMBINED  
DOYLE LAKE STATIONS, SPRING AND FALL, 1975

|  | Total Solids | Volatile Solids | Chemical Oxygen Demand | Total Kjeldahl Nitrogen | Total Phosphate | Number of Samples   |
|--|--------------|-----------------|------------------------|-------------------------|-----------------|---------------------|
| (Values in percent,<br>dry weight)                       |              |                 |                        |                         |                 |                     |
| Objectable <sup>(a)</sup><br>Sediment<br>Characteristics | >6.0         |                 | >5.0                   |                         | >1000           |                     |
| Polluted Sediments<br>"Light" <sup>(b)</sup>             |              | <5.0            |                        | <4.0                    |                 | <300 <sup>(c)</sup> |
| "Heavy"  |              | >8.0            |                        | >12.0                   |                 | >900                |
| Doyle Lake   |              |                 |                        |                         |                 |                     |
| Mean   | 50.0         | 4.03            | 5.85                   | 1446                    | 110             | 116                 |
| S  | 9.3          | 0.33            | 2.13                   | 221                     | 535             |                     |
| Max  | 64.0         | 4.70            | 10.50                  | 1650                    | 1980            |                     |
| Min  | 31.4         | 3.66            | 2.97                   | 810                     | 64              |                     |

(a) Selected bulk analysis allowable sediment constituents (National Science Foundation, 1973).

(b) Selected bulk analysis classification of polluted sediments (Corps of Engineers, 1970).

(c) Originally reported as P.

Doyle Lake Munitions Constituent Analysis

Water. The Doyle Lake effluent stream contained munitions concentrations similar to those of the production lines effluent stream (Grant Creek). However, significant TNT concentrations (Table 26) were detected only at the outfall and DL-1 water stations. Doyle Lake outfall (DL-0) values in spring samples were uniformly about 24 ppb while DL-1 water values fluctuated from 3.5 to 65.5 ppb. In the remaining water stations, TNT levels were much lower, being near or below the lower limit of accuracy of the analytical method (0.6 ppb). TNT values in DL-0 samples in the fall also ranged consistently above about 30 ppb, while the subsequent downstream water stations maintained concentrations of less than 0.6 ppb.

DNT values were much lower but detectable at all stations. Values were generally in the range of 1.0 ppb. Values for 2,6 DNT were below detection limits at all stations in the fall. Mean values and ranges of all munitions measured are presented in Table 26.

Sediment. The concentrations of munitions in sediment samples from Doyle Lake stations displayed some random fluctuations, but over a rather limited range (Table 27). Although some accumulation appeared to have occurred within the spring samples, the TNT concentrations here averaged less than 25 ppb with fairly uniform behavior exhibited by all stations in a particular day. However, limited fall data indicate an apparent fall-off of TNT concentrations from 42.9 ppb at DL-1 to less than 10 ppb in the subsequent stations. DNT values were usually quite low during both sampling periods, 2,4-DNT levels (average 21 ppb) appear to run somewhat higher than the 2,6-DNT values (average 4 ppb), as might be anticipated due to the greater photolytic stability of the 2,4 isomer.

Results of munitions compounds analyses on Doyle Lake sediments from spring and fall surveys appear in Appendix B, Tables 1 and 2.

TABLE 26. MEAN CONCENTRATIONS (RANGES) OF TNT, 2,4 DNT AND 2,6 DNT IN WATER SAMPLES FROM DOYLE LAKE

|                     | TNT (ppb)     | 2,4 DNT (ppb)  | 2,6 DNT (ppb)  |
|---------------------|---------------|----------------|----------------|
| <b>Spring, 1975</b> |               |                |                |
| DL-0                | 24 (7.0-31.0) | 1.1 (<0.2-2.7) | 0.8 (0.6-0.9)  |
| DL-1                | 28 (3.5-65.5) | 0.3 (<0.2-0.3) | 0.7 (<0.2-1.3) |
| DL-2                | <0.6          | 0.2 (<0.2-0.3) | 0.6 (<0.4-1.0) |
| DL-3                | <0.6          | 0.2 (<0.2-0.2) | 0.8 (<0.4-1.2) |
| DL-4                | <0.6          | 1.8 (<0.2-4.1) | 0.9 (<0.4-1.4) |
| <b>Fall, 1975</b>   |               |                |                |
| DL-0                | 40.1          | <0.2           | <0.4           |
| DL-1                | 43 (2.8-83.6) | 6.5 (3.2-9.7)  | <0.4           |
| DL-2                | <0.6          | 0.5 (0.4-0.6)  | <0.4           |
| DL-3                | <0.6          | <0.2           | <0.4           |
| DL-4                | <0.6          | 0.3 (<0.2-0.3) | <0.4           |

TABLE 27. MEAN CONCENTRATIONS (RANGES) OF TNT, 2,4 DNT AND 2,6 DNT IN SEDIMENT SAMPLES FROM DOYLE LAKE

|                     | TNT (ppb)      | 2,4 DNT (ppb)    | 2,6 DNT (ppb)  |
|---------------------|----------------|------------------|----------------|
| <b>Spring, 1975</b> |                |                  |                |
| DL-1                | 22 (<3.0-40.1) | 4.7 (3.2- 6.1)   | <0.2           |
| DL-2                | 10 (<3.0-17.3) | 8.7 (2.0-15.3)   | 3.9 (<2.0-5.7) |
| DL-3                | 17 (<3.0-30.7) | 23.8 (12.1-33.4) | 2.7 (<2.0-3.3) |
| DL-4                | 23 (3.4-42.7)  | 53.4 (6.8-100)   | 4.9 (3.9-5.8)  |
| <b>Fall, 1975</b>   |                |                  |                |
| DL-1                | 42.9           | 9.1              | <0.2           |
| DL-2                | <0.2           | 35.7             | 10.4           |
| DL-3                | 1.8            | 7.6              | <0.2           |
| DL-4                | 7.3            | 20.4             | <0.2           |

Doyle Lake Algae (Spring)

Discussions of Doyle Lake algal communities will concentrate on comparisons between similar stations, i.e., the periphyton of stream stations DL-1 and DL-4 and the phytoplankton of lake stations DL-2 and DL-3 will be compared.

Species Composition. Very few periphyton species were found on natural substrates in the effluent canal to Doyle Lake (DL-1, Appendix C, Table 25), in comparison to the outlet station (DL-4, Appendix C, Table 28). The species present at DL-1 were of the most tolerant genera, Euglena, Navicula, and Nitzschia (Palmer, 1969). Single-celled flagellates, coccoid green and blue-green algal species and a very tolerant diatom species, Navicula cryptocephala, were dominant. The low number of species plus the dominance by a few organisms reflects a situation of extreme perturbation at DL-1.

Conditions appeared more favorable for periphytic growth on natural substrates at DL-4. However, tolerant species were still present and the number of dominant species few.

Similar trends were shown by the data from artificial substrates. Twenty-three species with two being dominant were found at DL-1. Improvement was seen at DL-4 with a total of 59 species collected. Primarily tolerant species--Cyclotella meneghenia, Gomphonema parvulum, Nitzschia amphibia, N. palea, Scenedesmus quadricauda (Palmer, 1959 and 1969)--were dominant. Data from the artificial substrates for DL-1 and DL-4 appear in Appendix C, Tables 29 and 32, respectively.

Slight differences in the phytoplankton compositions of the shoreline (DL-2) and middle Doyle Lake (DL-3) stations occurred during the spring sampling (Appendix C, Tables 26 and 27). Three species--a single-celled flagellate, a coccoid green species, and Dinobryon divergens--were strongly dominant at both sites. Several other species also occurred in relative abundance at both stations. Those species not common to both stations occurred only infrequently in the samples.

Artificial substrate samplers again reflected similar species compositions between the two sites (Appendix C, Tables 30 and 31). A slightly higher number of species was found at DL-3, but the dominant organisms were the same at both stations. Several of the dominants were highly tolerant species--Gomphonema parvulum, Melosira varians, Nitzschia palea, Scenedesmus quadricauda, var. longispira, in particular (Palmer, 1959 and 1969).

Natural substrate periphyton and phytoplankton and artificial substrate periphyton data are summarized in Appendix C, Tables 18 and 24, respectively.

Community Structure. The natural substrates at DL-1 maintained a generally depauperate periphyton community during the spring survey. Coccoid green and blue-green species and single-celled flagellates almost totally dominated the flora. This over-abundance of a few species and low species diversity reflect a situation of extreme perturbation. A slight improvement was noted in the periphyton community at the Doyle Lake outfall, DL-4 (Table 28). The increases in community parameters may reflect the morphological differences between the two sites as well as any lessening of toxic effects of the LAP effluent by dilution. The same three organisms were predominant at DL-4 as at DL-1. In addition, abundant populations of two tolerant diatoms, Navicula cryptocephala and Stephanodiscus astraea, also occurred at DL-4. This community also showed signs of an environmental stress.

TABLE 28. MEANS OF NATURAL SUBSTRATE PERIPHYTON AND PHYTOPLANKTON DATA FROM DOYLE LAKE, SPRING, 1975

|      | Mean Number of Individuals x 10 <sup>5</sup> | Mean Number of Species | Mean Species Diversity |
|------|--|------------------------|------------------------|
| DL-1 | 1.1  | 5.8                    | 0.78                   |
| DL-4 | 1.3  | 11.8                   | 1.50                   |
| DL-2 | 1.7  | 8.8                    | 1.49                   |
| DL-3 | 2.0  | 8.6                    | 1.22                   |

Species-area curves of the periphyton data from the Doyle Lake natural substrates are presented in Figure 12. DL-1 appeared to have reached its climax community at the time of sampling. The community was very depauperate in comparison to that at DL-4. Morphological differences may have been responsible for the difference in the number of species. However, the very low number of species present at DL-1 and the composition of those species, coupled with the overall low diversities at both stations, indicates an external environmental stress.

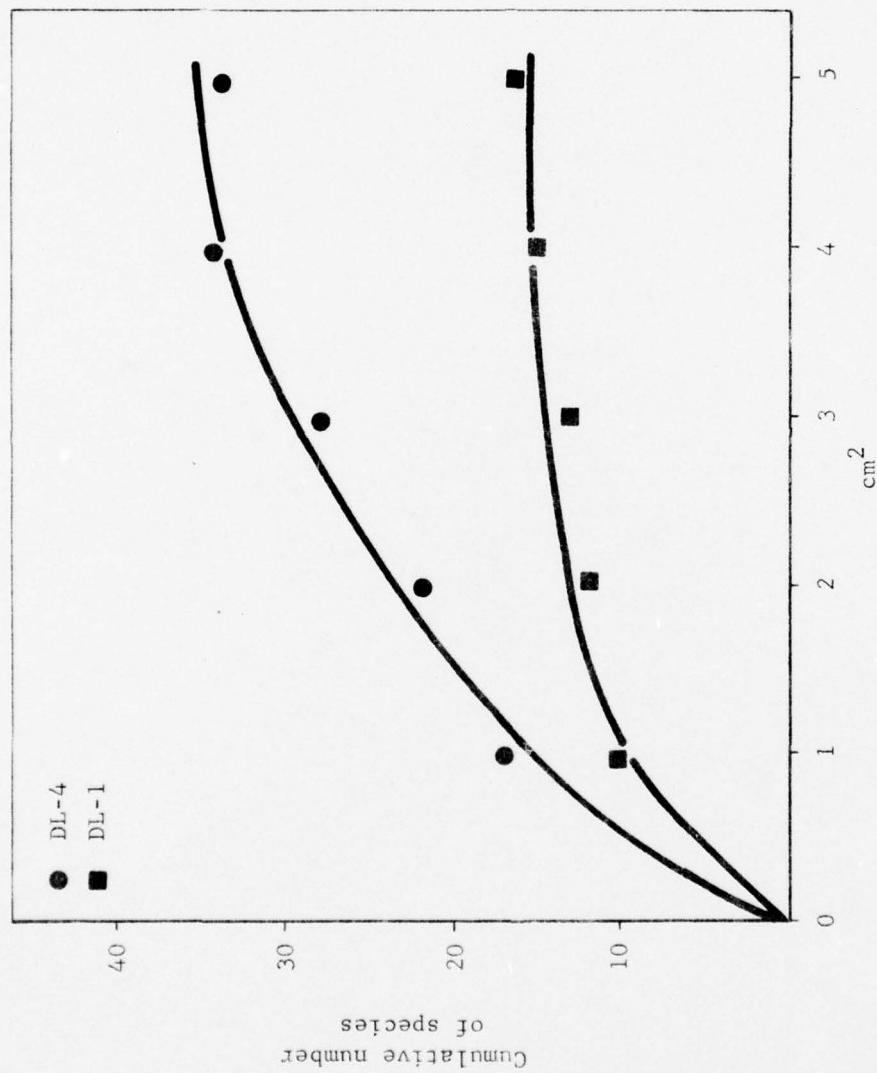


FIGURE 12. SPECIES-AREA CURVE FROM DOYLE LAKE PERIPHYTON DATA (NATURAL SUBSTRATES), SPRING, 1975

Artificial substrate periphyton samples from DL-1 showed community structures similar to those on the natural substrates. All three community parameters (shown in Table 29) were very low during the entire 3-week incubation period. The outfall from Doyle Lake, DL-4, showed a comparatively healthy community colonizing the artificial substrates. Dominance was shared by a large number of species, although a tolerant diatom, Gomphonema parvulum, was most abundant. Species diversities were relatively high as were the numbers of species. These results are inconsistent with those obtained from the natural substrate data at DL-4, which indicated perturbation.

Little difference was seen between either the number of individuals or the kinds of species present in the phytoplankton communities at DL-2 and DL-3 (Table 28). With the exception of one sample at DL-3, there was little variation between the low numbers of species found in each replicate. Figure 13, species-area curves for Doyle Lake, illustrates the comparison between DL-2 and DL-3 phytoplankton communities. The curves show a slightly higher number of species at DL-3. However, extrapolation of both curves indicates a convergence at approximately equal levels. Identical patterns were seen in the artificial substrate periphyton samples from the lake stations. Similar tolerant species were dominant at both stations-- Gomphonema parvulum, Melosira varians, Nitzschia palea. A greater number of species was present at DL-3, but the overall total number of individuals was comparable to DL-2. The additional species found at DL-3 were present in such low abundance as to affect the overall diversity for the station very little (Table 28).

Biomass, Chlorophyll a, and Autotrophic Index. Artificial substrates provided the samples used for biomass and chlorophyll a determinations. Autotrophic indices, calculated from the ratio of biomass to chlorophyll a, indicate extreme stress at DL-1 which was reduced considerably at DL-4 (Table 30). A value of 100 serves as the standard to which comparisons are made. An index greater than 100 is indicative of perturbation [specifically of organic pollution (Weber and McFarland, 1969)].

TABLE 29. MEANS OF ARTIFICIAL SUBSTRATE PERiphyton DATA  
FROM DOYLE LAKE, SPRING, 1975

|      | Week 1                                      |                           |                           |  | Week 2                                      |                           |                           |  | Week 3                                      |                           |                           |  |
|------|---|---------------------------|---------------------------|--|---|---------------------------|---------------------------|--|---|---------------------------|---------------------------|--|
|      | Mean Number<br>of Individuals $\times 10^5$ | Mean Number<br>of Species | Mean Species<br>Diversity |  | Mean Number<br>of Individuals $\times 10^5$ | Mean Number<br>of Species | Mean Species<br>Diversity |  | Mean Number<br>of Individuals $\times 10^5$ | Mean Number<br>of Species | Mean Species<br>Diversity |  |
| DL-1 | 0.1   | 10                        | 1.57                      |  | 0.1   | 8                         | 1.27                      |  | .06   | 6                         | 1.61                      |  |
| DL-4 | 1.0   | 29                        | 2.51                      |  | 1.4   | 28.3                      | 2.84                      |  | 2.7   | 30                        | 2.83                      |  |
| DL-2 | 1.2   | 20                        | 2.08                      |  | 1.2   | 30.3                      | 2.67                      |  | 1.9   | 18                        | 2.42                      |  |
| DL-3 | 0.8   | 12                        | 1.98                      |  | 1.8   | 36                        | 2.73                      |  | 0.8   | 18                        | 2.37                      |  |

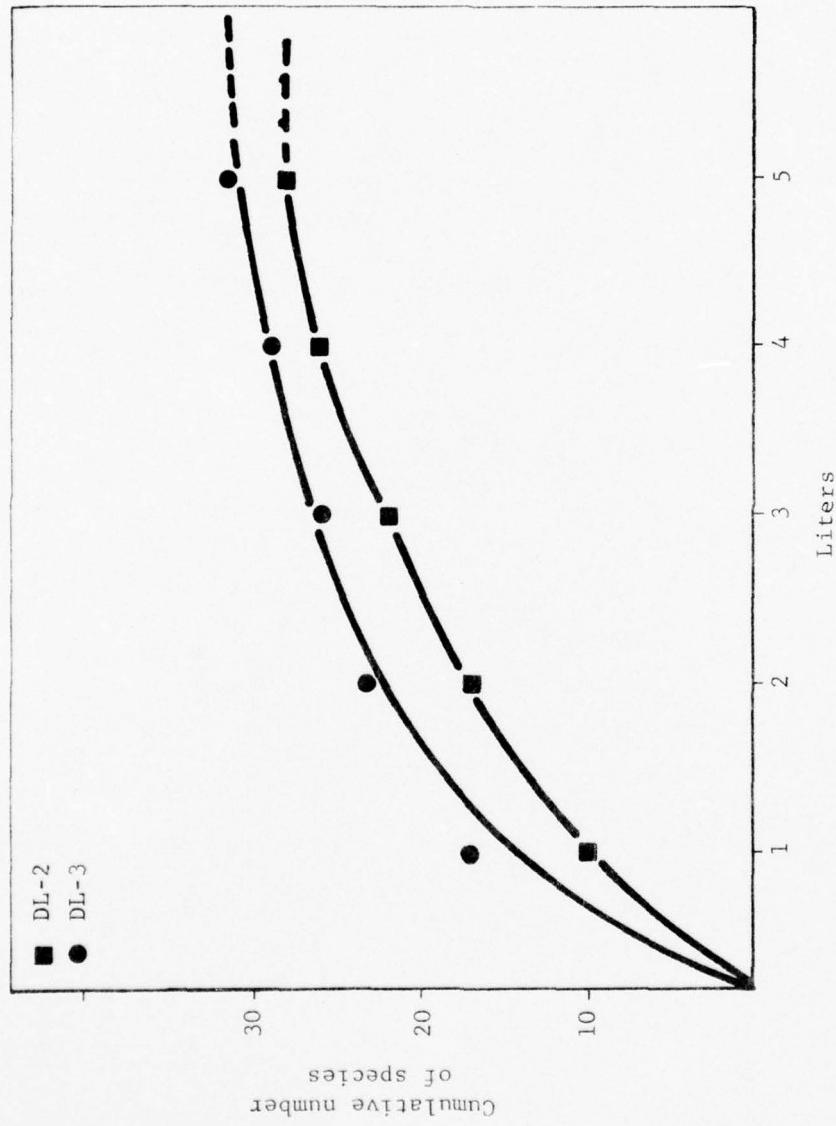


FIGURE 13. SPECIES AREA CURVES FROM DOYLE LAKE PHYTOPLANKTON DATA, SPRING, 1975

TABLE 30. BIOMASS, CHLOROPHYLL a, AND AUTOTROPHIC INDICES FOR DOYLE LAKE, SPRING, 1975

|      | Biomass<br>mg/m <sup>2</sup> | Chlorophyll <u>a</u><br>mg/m <sup>2</sup> | Autotrophic Index<br>Biomass/Chl <u>a</u> |
|------|------------------------------|---|---|
| DL-1 | 521.0                        | 0.76                                      | 689.2                                     |
| DL-4 | 3831.9                       | 30.16                                     | 127.1                                     |
| DL-2 | 7349.5                       | 31.42                                     | 234.0                                     |
| DL-3 | 2488.6                       | 19.79                                     | 125.8                                     |

Biomass, chlorophyll a, and resultant index were higher at DL-2 than at DL-3 (Table 29). Both indices were greater than 100 although DL-3 approaches that value. No reason for the greater amount of non-autotrophic matter is readily evident from the data (Appendix C, Tables 30 and 31). The DL-2 station was located nearer a source of organic enrichment (the LAP effluent) than DL-3. Substantial settling or dilution may have occurred at DL-3. The similarity of the indices of DL-3 and DL-4 indicates that effects from enrichment did not extend much beyond DL-2.

Colonization. Colonization of the artificial substrate samplers was complete at DL-1 within one week of incubation (Table 29). The periphytic community at DL-4 however had not reached its peak at week three when the size of the existing population nearly doubled over that of the second week. Colonization by the largest number of species occurred during the second week at DL-2. By the third week, many species had disappeared allowing large population increases by more competitive species, resulting in an overall increase in the number of organisms. This increase was not seen at DL-3 where colonization was complete at week two.

#### Doyle Lake Algae (Fall)

Species Composition. The kinds and numbers of periphyton species found on the natural substrates at the Doyle Lake stream stations (DL-1 and

DL-4) indicate a perturbation in the effluent canal to Doyle Lake which is somewhat lessened by dilution at the outfall (DL-4). Species present at DL-1 were of tolerant types; dominance was exhibited by four species--Navicula minima, Nitzschia amphibia, coccoid green and a blue-green species. At DL-4 the species were again of generally tolerant forms but dominance was shared by more species and was less pronounced than at DL-1. The most abundant species were Spirogyra sp., coccoid green and blue-green species and a filamentous blue-green species.

Data from the artificial substrates are inconclusive. Flows during the fall survey were very low. As a result, the diatomometer at DL-1 was submerged for only one week. During that time, only six species in very low numbers had colonized the sampler. Low flows also affected DL-4 by causing the outfall to become a stagnant pool. The increase in pond-type species (Oedogonium, Scenedesmus, Spirogyra, Ulothrix, Euglena), and the low number of predominantly tolerant species bare evidence to this fact.

A larger number of periphyton species were found on the artificial substrates at DL-2 than at DL-3. Complete mixing of the effluent in the lake may not have occurred due to low flows resulting in differences in water quality between the shoreline and middle stations. High nitrates at DL-2 were probably responsible for differences in algal populations. Dominant species were similar at both stations and consisted of primarily tolerant species--Cyclotella meneghiniana, Melosira distans, Navicula cryptocephala, Nitzschia acicularis, N. closterium, N. palea, and Scenedesmus quadricauda.

Phytoplankton species were found to be fairly similar at both lake stations. Dominant species at both sites were Kirchneriella sp., coccoid green and blue-green species, Merismopedia sp., and unicellular flagellates. Species were generally very tolerant types and dominance was evident in only a few species, indicating a stressed algal community.

Natural substrate periphyton, phytoplankton, and artificial periphyton data from all Doyle Lake stations appear in Appendix C, Tables 33 through 40.

Community Structure. Large numbers of only a few species and an overall low species diversity found at DL-1 on the natural substrates indicate

an area of pronounced stress (Table 31). Dilution from Doyle Lake may have served to diminish effects of any introduced toxicants at the outfall, DL-4, as shown by the increased diversity. However, the diversity was altered mainly by increases in chlorophycean and cyanophycean pond-type species populations. These increases likely reflected the habitat alteration due to low flows as well as effects due to water quality changes.

TABLE 31. MEANS OF NATURAL SUBSTRATE PERIPHYTON AND PHYTOPLANKTON DATA FROM DOYLE LAKE, FALL, 1975

|      | Mean Number of<br>Individuals $\times$<br>$10^5$ | Mean Number of<br>Species | Mean Species<br>Diversity |
|------|--|---------------------------|---------------------------|
| DL-1 | 2.3  | 9.8                       | 1.37                      |
| DL-4 | 3.1  | 15.4                      | 2.02                      |
| DL-2 | 10.1   | 14.0                      | 1.69                      |
| DL-3 | 8.4  | 11.4                      | 1.56                      |

Species area curves plotted from the periphyton data from the natural substrates at DL-1 and DL-4 appear in Figure 14. Sufficient analyses were performed to adequately describe both algal communities. The difference in number of species may illustrate the morphological differences between the two stations as well as possible differences in water quality.

Algal communities found on artificial substrates were similarly affected during the first week of incubation (Table 32). Further comparisons were not possible as information for DL-1 could not be obtained due to low flows. The community at DL-4 was comprised primarily of tolerant pond species. Diatomometer slides were heavily colonized by Spirogyra sp. The predominance of only one species, even in the stagnant conditions found in the outfall area, indicates an external perturbation.

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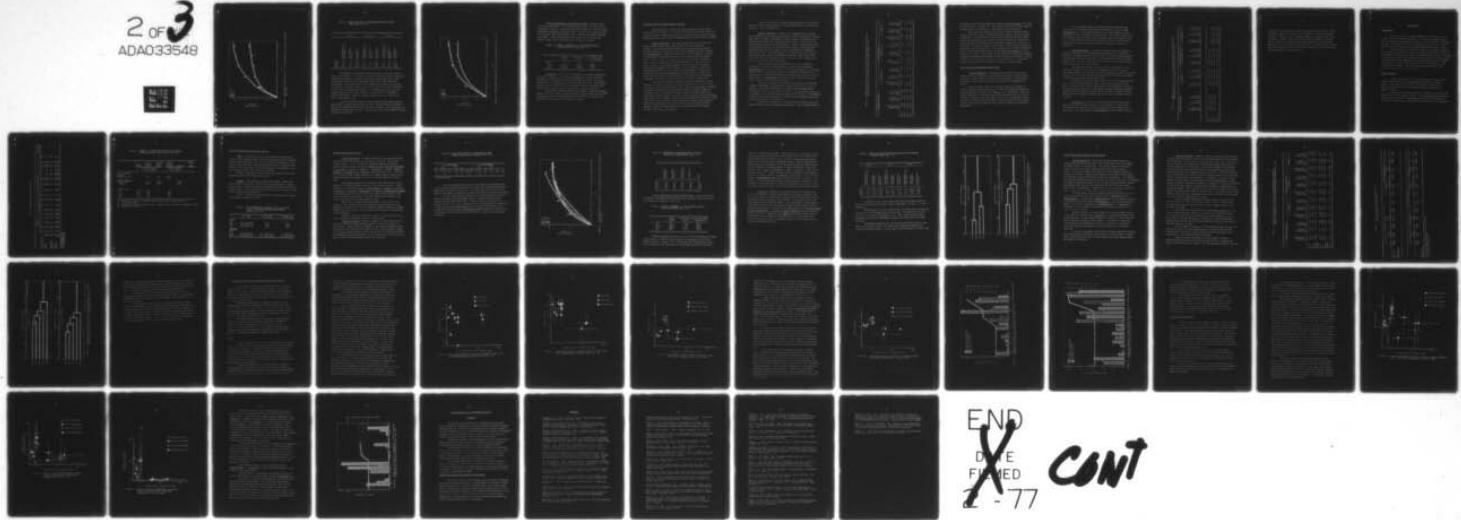
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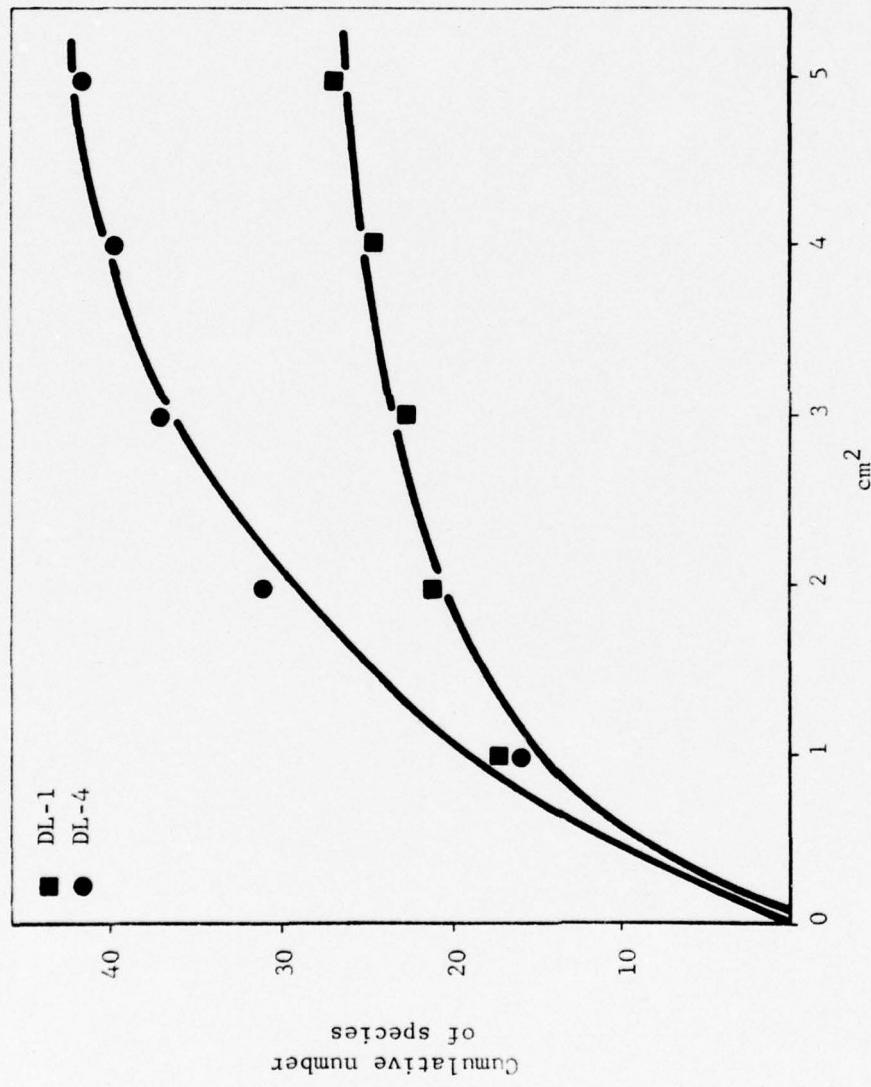


FIGURE 14. SPECIES-AREA CURVES FROM DOYLE LAKE PERIPHYTON DATA (NATURAL SUBSTRATES), FALL, 1975

TABLE 32. MEANS OF ARTIFICIAL SUBSTRATE PERIPHYTON DATA FROM  
DOYLE LAKE, FALL, 1975

|      | Week 1                                   |                        |                        | Week 2                                   |                        |                        | Week 3                                   |                        |                        |
|------|--|------------------------|------------------------|--|------------------------|------------------------|--|------------------------|------------------------|
|      | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity |
| DL-1 | 0.05                                     | 6                      | 1.67                   | --                                       | --                     | --                     | --                                       | --                     | --                     |
| DL-4 | 1.8                                      | 13                     | 1.90                   | 10.1                                     | 13                     | 0.62                   | 7.2                                      | 17                     | 1.33                   |
| DL-2 | 2.2                                      | 23                     | 2.61                   | 4.0                                      | 19                     | 2.69                   | 3.9                                      | 23                     | 2.80                   |
| DL-3 | 0.7                                      | 11                     | 1.75                   | 1.8                                      | 13                     | 2.42                   | 4.7                                      | 20                     | 2.58                   |

Phytoplankton investigations showed similarity in the major species found at DL-2 and DL-3. Green and blue-green species and unicellular flagellates comprised the dominants. A larger total number of species was found at DL-2 the shoreline station, than at the middle station, DL-3. The statistical significance of this difference was not determined as the data were unsuitable for analyses of variance. Species not common to both stations were represented by only one or two specimens per station. The standing crop at DL-2 was also slightly higher than DL-3 which indicates a possible source of enrichment from the LAP effluent. Figure 15 shows the species-area curves constructed from the phytoplankton data.

Artificial substrates were colonized similarly by periphytic algae at DL-2 and DL-3. Dominant species were identical. The differences in numbers of species and individuals shown in Table 32 are attributable to the small number of analyses performed on DL-3 samplers. Proportions of algal groups appeared constant between the two stations.

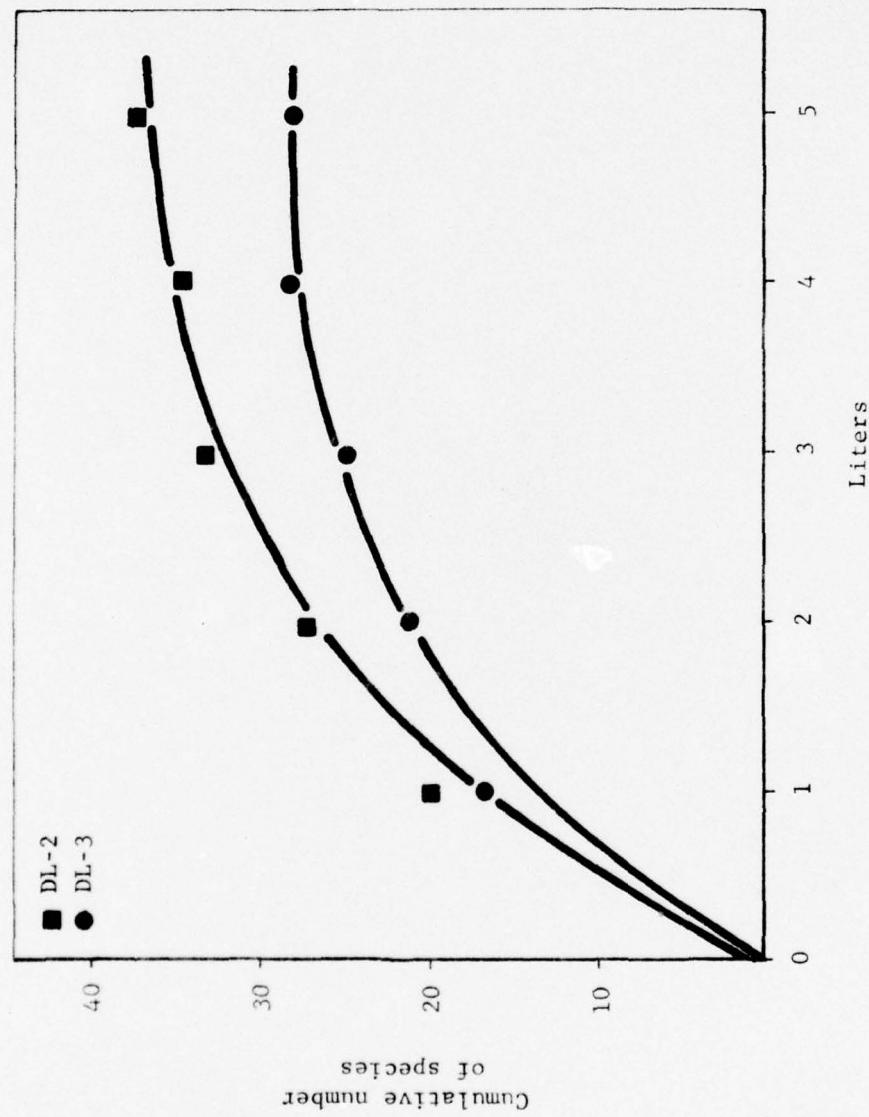


FIGURE 15. SPECIES-AREA CURVES FROM DOYLE LAKE PHYTOPLANKTON DATA, FALL, 1975

Biomass, Chlorophyll a, and Autotrophic Index. Biomass of algae colonizing the diatometer slides was excessive in relation to the amount of chlorophyll a present at all Doyle Lake stations (Table 33). DL-4 most nearly approaches an acceptable autotrophic index of 100 but still showed some sign of perturbation (Weber and McFarland, 1969). DL-2 and DL-3 showed comparable high indices. The excessive biomass may have been due to heterotrophic bacteria, fungi, and/or dead algal masses at these two stations.

TABLE 33. BIOMASS, CHLOROPHYLL a, AND AUTOTROPHIC INDICES FROM DOYLE LAKE STATIONS, FALL, 1975

|      | Biomass<br>mg/m <sup>2</sup> | Chlorophyll a<br>mg/m <sup>2</sup> | Autotrophic Index<br>Biomass/Chl <u>a</u> |
|------|------------------------------|------------------------------------|---|
| DL-1 | --                           | --                                 | --  |
| DL-4 | 4,895.5                      | 16.30                              | 158.6                                     |
| DL-2 | 12,434.3                     | 16.03                              | 762.8                                     |
| DL-3 | 11,564.6                     | 30.86                              | 721.5                                     |

Colonization. The numbers of organisms colonizing the diatometers increased by a factor of 10 at DL-4 between weeks one and two (Table 32). Sloughing occurred during the third week allowing more species to colonize. Heaviest growths of Spirogyra sp. occurred during this period. (No comparison with the rate of a similar area receiving higher effluent concentration was possible as the station providing that information was intermittent.)

DL-2 and DL-3 showed a doubling of the number of individuals between weeks one and two; at week three DL-2 maintained both the number of species and individuals of week 2; at week three DL-3 again doubled in the size of its populations and increased in total number of species.

Doyle Lake Benthic Macroinvertebrates (Spring)

Sampling stations in the area of Doyle Lake were located in both stream and pond type habitats. Discussions of the macroinvertebrate communities of these areas will compare and contrast DL-1 (effluent canal) with DL-4 (outlet from Doyle Lake); and DL-2 and DL-3 (pond habitats) with similar aquatic habitats.

Species Composition. The species compositions of the benthic communities inhabiting the areas of DL-1 and DL-4 are quite different. A variety of invertebrates from many orders were collected at DL-4 from both natural and artificial substrates. The two most abundant organisms collected were Glyptotendipes sp. (never collected at DL-1) and oligochaetes. However, no such variety of organisms was found at DL-1 located in the LAP effluent canal. At this station, a few species (5) of Dipteran larvae, a dragon fly naiad, a beetle, a species of snail and oligochaetes were the only invertebrates collected. The dominant organisms at this station were the highly tolerant snail, Physa sp. and oligochaetes. This low number of species (for stream habitats) found at DL-1 coupled with the dominance of tolerant organisms would indicate an environmentally stressed situation. Conditions at DL-4 are greatly improved, with the number of species found here approaching or exceeding the numbers collected from control stations in Grant Creek.

The biological community inhabiting the natural substrates of Doyle Lake (DL-2 and DL-3) is composed of organisms typically found in soft, silty substrates of lakes and ponds (Hynes, 1970). Two groups which might be expected to occur here but were not collected are Pelecypods (freshwater clams) and burrowing mayflies. The absence of these organisms from small ponds is not particularly unusual, however. Artificial substrate samples collected from these stations had a larger number of species (22 compared with only 8 from the natural substrates). This situation would be expected as the soft, silty substrates provide suitable habitats for very few organisms as previously mentioned. However, a variety of organisms are able to colonize the numerous niches provided by multiple plate samplers.

Complete species lists of benthic macroinvertebrates collected from natural and artificial substrates at each Doyle Lake station are presented in Appendix D, Tables 25 through 32.

Community Structure. A review of the community structure parameters presented in Table 34 shows the benthic community inhabiting the effluent canal (DL-1) to have a lower number of species, number of individuals, and species diversity than the outlet stream station (DL-4). This was true for both natural and artificial substrate samples. The most striking observation in the natural substrate data is the much lower number of individuals per sample collected from DL-1--a mean of only 8.4 individuals as compared to 101.4 found at DL-4. This same trend of low standing crops at DL-1 was also demonstrated by the artificial substrate collections. These results indicate the presence of a depauperate benthic community at DL-1 probably due to the poor water quality of the LAP effluent.

Recovery from the stress witnessed at DL-1 is not complete at station DL-4 as 50% of all the organisms collected were of two species, Glyptotendipes sp. and an oligochaete. This large percentage of only two organisms, one known to be pollution tolerant (oligochaeta) indicates that an environmental stress was still being exerted on the benthic community at DL-4. Large numbers of Glyptotendipes sp. were also found on the artificial substrates causing low species diversity indices.

Sampling of the natural substrates at DL-2 and DL-3 in Doyle Lake produced a mean number of individuals per sample (0.09 sq. m.) of 34.0. A relatively few species (mean=2.60) were collected and were predominantly oligochaetes, which gave low diversity indices. This type benthic community is not uncommon in the soft silty bottom sediments of small lakes. These sediments are quite uniform and provide relatively few types of microhabitats which can be colonized by benthic invertebrates.

Artificial substrates set in Doyle Lake were colonized by many more organisms than the natural substrates could support. Again the high number of

TABLE 34. SUMMARY OF ANALYSES OF THE BENTHIC-MACRO INVERTEBRATE DATA FROM NATURAL AND ARTIFICIAL SUBSTRATE SAMPLES FROM DOYLE LAKE, SPRING, 1975

one species (over 70% of all organisms collected were Glyptotendipes sp.) caused diversity indices to be quite low. Here again, as at DL-4, this large number of a single species is indicative of an environmentally stressed system.

Due to the small number of stations and a lack of adequate control stations, statistical analysis (ANOVA and cluster analysis) were not performed on these data. Colonization rate plots from the artificial substrate sample data are not presented as they provide very limited additional information as to the condition of the benthic macroinvertebrate communities in the area of Doyle Lake. The rate of increase in both numbers of species and individuals is much greater at DL-4 than at DL-1. This is again due to the rapid colonization at DL-4 of the multiple-plate samplers by the midge Glyptotendipes sp. which did not occur at DL-1. The situation at DL-2 and DL-3 was one where the benthic community had reached maximum numbers by the third week and had begun to decline by week four. Due to the lack of specific control stations, no conclusions concerning colonization rate variations from the normal can be drawn.

#### Doyle Lake Benthic Macroinvertebrates (Fall)

Species Composition. The benthic macroinvertebrate communities inhabiting the areas sampled around Doyle Lake during the fall were composed of groups of species similar to those found in the spring. Notable differences were a lower number of Diptera species and only one Ephemeroptera species.

The two stream stations, DL-1 and DL-4, had similar numbers of species, with DL-4 having slightly more. However, the dominant organisms at both stations were a tolerant snail, Physa sp., and oligochaetes. The invertebrate communities of both these areas were influenced by the low flows at the time of sampling. The area sampled at DL-4 was an isolated pool created by the lack of overflow from Doyle Lake. Dissolved oxygen levels fluctuated greatly in this pool and conditions there were not optimal for invertebrate colonization.

The invertebrate community colonizing artificial substrate samplers in Doyle Lake proper was completely dominated by a species of midge, Glyptotendipes sp.. This complete dominance by a single organism is often indicative of an environmentally stressed habitat. This stress condition was most probably created by the low flow causing the lake to be stagnant at this time. Complete data sets for both natural and artificial substrate samples from Doyle Lake during the fall are presented in Appendix D, Tables 33 through 40.

Community Structure. A summary of community structure parameters calculated from samples collected from the Doyle Lake area is presented in Table 35. Collections from the stream stations consistently had lower numbers of individuals than the lake stations. The values from the natural substrate samples at DL-1 were the lowest recorded and probably reflect low flow conditions as well as munitions waste effects.

The number of individuals colonizing artificial substrates at stations DL-2 and DL-3 was very high but was composed of mainly one species. These large numbers of one organism resulted in exceedingly low species diversity indices. All values were less than 1.0, characteristic of aquatic communities in environmentally stressed areas (Wilhm, 1970).

In general, the effluent canal at DL-1 was a severely stressed system due to both low flow and munitions wastes. Some recovery occurred at DL-4 after a retention period in Doyle Lake; however, an environmental perturbation was still evident at this site. Conditions in the lake itself (DL-2 and DL-3) resembled those of slightly polluted areas but the lack of adequate controls makes conclusive statements concerning the state of the invertebrate community difficult.

Colonization. Rates of colonization of artificial substrate samplers placed in Doyle Lake were again, as in the spring, of little benefit in evaluating the condition of the benthic macroinvertebrate communities of these areas. The large numbers of Glyptotendipes sp. which rapidly colonized

TABLE 35. SUMMARY OF ANALYSES OF THE BENTHIC-MACROINVERTEBRATE DATA FROM NATURAL AND ARTIFICIAL SUBSTRATE SAMPLES FROM DOYLE LAKE, FALL, 1975

| Natural Substrates         | Artificial Substrates  |                        |                        |
|----------------------------|------------------------|------------------------|------------------------|
|                            | Week 2                 | Week 3                 | Week 4                 |
| Mean Number of Individuals | Mean Number of Species | Mean Number of Species | Mean Number of Species |
| Diversity                  | Diversity              | Diversity              | Diversity              |
| DL-1                       | 8.20                   | 2.40                   | 0.55                   |
| DL-2                       | 4.00                   | 0.80                   | 0.00                   |
| DL-3                       | 12.00                  | 2.00                   | 0.60                   |
| DL-4                       |                        |                        |                        |
| 1278.50                    | 4.50                   | 0.62                   | 3918.00                |
| 865.50                     | 5.00                   | 0.33                   | 1973.67                |
| 32.00                      | 4.00                   | 0.65                   | 35.00                  |
|                            |                        |                        | 5.00                   |
|                            |                        |                        | 1.14                   |
|                            |                        |                        | 22.00                  |
|                            |                        |                        | 4.00                   |
|                            |                        |                        | 1.19                   |
| 199.00                     | 5.00                   | 0.27                   | 3353.00                |
| 2.67                       | 0.01                   |                        | 4.00                   |
| 2.33                       | 0.08                   |                        | 0.05                   |
| 1.14                       |                        |                        | 1376.50                |
|                            |                        |                        | 6.00                   |
|                            |                        |                        | 0.29                   |

the samplers at DL-2 and DL-3 produced high rates for these stations. Rates at the two stream stations appeared to be similar at the end of two weeks' incubation. However, after three weeks, numbers of individuals and species continued to increase at DL-1, while colonization of samplers at DL-4 had ceased. A decline in numbers was observed by the end of the fourth week. The slow rate of colonization at DL-4 (Appendix D, Table 40) was most probably due to the stagnant condition of the water, creating a very low invertebrate-carrying capacity at this time of year.

Prairie CreekWater Quality

Results of the water chemistry analyses for the fall survey of Prairie Creek are summarized in Table 36. Selected water quality criteria are also presented for comparison. Sampling station locations are illustrated on Figure 1; descriptions of sites are included in Table 1. The complete data set from which the summary was composed appears in Appendix A, Table 2.

The water quality of the upstream Prairie Creek stations (PC-1 and PC-2) compares well with the control station data from Grant Creek (Table 3). The somewhat elevated dissolved solids concentrations upstream (mean = 602 mg/l) are further increased at the confluence of the LAP effluent stream with monitored TDS values greater 1000 mg/l. This waste discharge (PC-0) also seems to contribute to Prairie Creek's already nutrient enriched nature. Downstream from the discharge, both nitrogen and phosphorus species exceed desirable criteria by a factor of 3 to 4.

Sediment Chemistry

Sediment characteristics of the combined Prairie Creek stations downstream of the LAP effluent are summarized in Table 37. Polluted sediment composition is also included for reference. Results of all analyses performed on Prairie Creek sediment samples are presented in Appendix B, Table 2.

From the data presented in Table 37, it appears that no objectionable levels for either volatile solids or total Kjeldahl nitrogen are being approached in the sediments. This implies no accumulation of substances from the LAP effluent and an unpolluted situation, by the proposed standards.

TABLE 36. WATER QUALITY DATA FROM PRAIRIE CREEK, FALL, 1975

| Field Measurements                      | Laboratory Measurements (ppm) |       |           |      |            |             |              |         |         |         |                 | Number of Samples Considered |                 |                 |                 |      |     |       |
|---|-------------------------------|-------|-----------|------|------------|-------------|--------------|---------|---------|---------|-----------------|------------------------------|-----------------|-----------------|-----------------|------|-----|-------|
|   | pH                            | Cond. | Temp., °C | D.O. | Total Alk. | Susp. Hard. | Diss. Solids | COD (a) | TOC (b) | TKN (c) | NH <sub>4</sub> | NO <sub>2</sub>              | NO <sub>3</sub> | PO <sub>4</sub> | SO <sub>4</sub> | Cl   | Lab | Field |
|   | 6-9                           | <900  | -         | >5   | -          | <80         | <500         | <6.0    | <12     | <1.0    | <0.02           | -                            | <1.5            | <0.1            | <250            | <250 |     |       |
| Selected Criteria                       |                               |       |           |      |            |             |              |         |         |         |                 |                              |                 |                 |                 |      |     |       |
| Upstream<br>(PC-1,2)                    | Mean                          | 8.2   | 795       | 16.6 | 6.9        | 274         | 419          | 29      | 602     | 10      | 5.0             | 0.5                          | 0.07            | 0.26            | 162             | 8    | 4   | 10    |
|   | S                             | 0.3   | 47        | 1.3  | 1.7        | 12          | 30           | 18      | 49      | 5       | 0.5             | 0.1                          | 0.03            | 0.06            | 20              |      |     |       |
|   | Max                           | 8.6   | 875       | 18.9 | 9.0        | 292         | 458          | 54      | 644     | 15      | 6.0             | 0.6                          | 0.11            | <0.02           | <1.0            | 0.30 | 182 | 1     |
|   | Min                           | 7.5   | 740       | 15.0 | 5.1        | 266         | 394          | 14      | 550     | <4      | 5.0             | 0.3                          | 0.05            | 0.17            | 141             |      |     | 9     |
| Discharge to<br>Prairie Creek<br>(PC-0) | High                          | 8.1   | 1640      | 25.2 | 8.8        | 282         | 143          | 26      | 1044    | 12      | 3.0             | 0.4                          | 0.27            | 0.52            | 2.0             | 0.91 | 280 | 193   |
|   | Low                           | 7.8   | 1500      | 23.6 | 7.3        | 268         | 141          | 16      | 1036    | 10      | 3.0             | 0.3                          | 0.25            | 0.02            | 2.0             | 0.10 | 274 | 191   |
|   | Mean                          | 7.9   | 973       | 18.0 | 8.7        | 265         | 300          | 23      | 619     | 13      | 7.5             | 0.3                          | 0.07            | 0.04            | 1.3             | 0.35 | 166 | 62    |
|   | S                             | 0.3   | 223       | 1.8  | 3.0        | 45          | 44           | 12      | 128     | 6       | 3.5             | 0.1                          | 0.02            | 0.03            | 0.5             | 0.13 | 49  | 16    |
| Downstream<br>(PC-3,4,5)                | Max                           | 8.4   | 1150      | 22.0 | 13.4       | 290         | 336          | 38      | 736     | 19      | 11.0            | 0.4                          | 0.09            | 0.08            | 2.0             | 0.47 | 210 | 77    |
|   | Min                           | 7.2   | 320       | 15.9 | 5.4        | 198         | 247          | 10      | 490     | 5       | 4.0             | <0.2                         | <0.05           | <0.02           | <1.0            | 0.22 | 123 | 47    |

(a) Chemical Oxygen Demand

(b) Total Organic Carbon

(c) Total Kjeldahl Nitrogen

TABLE 37. SUMMARY OF SEDIMENT CHARACTERISTICS FOR COMBINED  
DOWNSTREAM PRAIRIE CREEK STATIONS, FALL, 1975

|   | Total Solids                       | Volatile Solids | Chemical Oxygen Demand | Total Kjeldahl Nitrogen        | Phosphate | Number of Samples   |
|---|------------------------------------|-----------------|------------------------|--------------------------------|-----------|---------------------|
|   | (Values in percent,<br>dry weight) |                 |                        | (Values in ppm,<br>dry weight) |           |                     |
| Objectionable <sup>(a)</sup><br>Sediment<br>Characteristics |                                    | >6.0            |                        | >5.0                           |           | >1000               |
| Polluted Sediments  |                                    |                 |                        |                                |           |                     |
| "Light" <sup>(b)</sup>                                      |                                    | <5.0            |                        | <4.0                           |           | <300 <sup>(c)</sup> |
| "Heavy"   |                                    | >8.0            |                        | >12.0                          |           | >900                |
| Prairie Creek:  |                                    |                 |                        |                                |           |                     |
| Mean  | 72.8                               | 2.33            |                        | 368                            |           | 8                   |
| S   | 8.5                                | 0.47            | -                      | 240                            | -         |                     |
| Max   | 83.5                               | 2.86            |                        | 700                            |           |                     |
| Min   | 63.2                               | 1.71            |                        | 147                            |           |                     |

(a) Selected bulk analysis allowable sediment constituents (National Science Foundation, 1973).

(b) Selected bulk analysis classification of polluted sediments (Corps of Engineers, 1970).

(c) Originally reported as P.

Prairie Creek Munitions Constituent Analysis

Water. Prairie Creek stations were sampled and analyzed only during the fall sampling period. From the limited data obtained it appears that the highest TNT water values of about 35 ppb were experienced at the outfall station (Table 38). The remaining water stations displayed concentrations of less than 10 ppb. DNT values were quite low throughout, being near or below our lower limit of accuracy (0.6 ppb).

Results of analyses of Prairie Creek water samples for munitions compounds are presented in Appendix A, Table 2.

Sediment. The analysis of Prairie Creek sediment samples indicated a maximum accumulation of TNT of about 40 ppb (Table 38). Average concentrations of 2,4-DNT (25 ppb) were somewhat higher than those found for 2,6-DNT (average 3 ppb), perhaps indicative of the relative stability of the 2,4-DNT to photolytic decomposition.

Results of munitions compound analyses for Prairie Creek sediments are presented in Appendix B, Table 2.

TABLE 38. MEAN CONCENTRATIONS (RANGES) OF TNT, 2,4 DNT AND 2,6 DNT IN PRAIRIE CREEK WATER AND SEDIMENT SAMPLES, FALL, 1975

|                  | TNT (ppb)      | 2,4 DNT (ppb)    | 2,6 DNT (ppb)  |
|------------------|----------------|------------------|----------------|
| <u>Water</u>     |                |                  |                |
| PC-0             | 35 (33.2-36.0) | <0.2             | <0.4           |
| PC-3             | <10 (<0.6-6.4) | <0.2             | <0.4           |
| PC-4             | <10 (<0.6-7.5) | <0.2             | <0.4           |
| <u>Sediments</u> |                |                  |                |
| PC-0             | --             | --               | --             |
| PC-3             | 11 (<2.0-26.3) | 23.7 (20.7-27.2) | 1.9 (<0.2-5.2) |
| PC-4             | 16 (<2.0-36.9) | 27.3 (5.3-48.3)  | 3.1 (<2.0-5.2) |

Prairie Creek Periphyton (Fall)

Species Composition. Periphyton occurring on the natural substrates in Prairie Creek was a mixture of clean water, facultative, and pollution-tolerant species. At PC-3, evidence of enrichment appeared as increased growth of several species, particularly Melosia varians, Navicula cryptocephala, N. graciloides, N. minima, N. radiosa, N. tripunctata, N. viridula, Nitzschia amphibia, N. apiculata, and N. fonticala, which persisted at PC-4. Other than the increases in these diatom populations, no change in species composition on natural substrates was apparent as a result of the JAAP effluent to Prairie Creek.

The periphyton data from the artificial substrates showed a similar response to the plant effluent as was seen on the natural substrates. Large populations of diatoms--M. varians, N. cryptocephala, N. radiosa, N. salinarum, N. amphibia, N. apiculata, M. microcephala, N. palea, and Stephanodiscus astraea--occurred immediately below the outfall at PC-3 and remained dominant at PC-4. Little other change attributable to the plant discharge was effected on the species compositions found on the diatometer slides.

Station PC-5, located furthest downstream in Prairie Creek, was intended to aid in the determination of recovery should an impact have been apparent at PC-3 and PC-4. As no such impact was observed, analyses of samples from PC-5 were only cursory. For that reason, PC-5 data will be excluded from further discussion.

Appendix C, Tables 41 through 50, present the natural and artificial substrate data from the periphyton study of Prairie Creek.

Community Structure. Periphyton communities on both the natural and artificial substrates showed identical responses to the plant effluent in Prairie Creek. Large increases in population sizes occurred immediately below the outfall at PC-3 and were still evident at PC-4. These increases in numbers of individuals showed upstream stations, PC-1 and PC-2, to differ significantly from downstream stations (Table 39). On natural substrates, the increase in the number of species occurring downstream was also significant.

TABLE 39. ANOVA FOR PERiphyton DATA FROM PRAIRIE CREEK,  
NATURAL AND ARTIFICIAL SUBSTRATES, FALL, 1975

| Station | Natural Substrates          |         |                         |         | $\bar{H}$ | Station | Artificial Substrates       |         |                         |         | $\bar{H}$ |
|---------|-----------------------------|---------|-------------------------|---------|-----------|---------|-----------------------------|---------|-------------------------|---------|-----------|
|         | Number<br>of<br>Individuals | Station | Number<br>of<br>Species | Station |           |         | Number<br>of<br>Individuals | Station | Number<br>of<br>Species | Station |           |
| PC1-2   | $4.7 \times 10^5$           | PC1-2   | 23.0                    | PC1-2   | 2.54      | PC1-2   | $2.6 \times 10^4$           | PC3-4   | 23.6                    | PC1-2   | 2.44      |
| PC3-4   | $1.0 \times 10^6$           | PC3-4   | 27.6                    | PC3-4   | 2.65      | PC3-4   | $9.0 \times 10^4$           | PC1-2   | 24.1                    | PC1-2   | 2.64      |
|         | .999                        |         | .90                     |         | —         |         | .99                         |         | —                       |         | —         |

— no significant difference

Study of the species-area curves constructed from the periphyton data from the natural substrates in Prairie Creek shows little difference between the communities at any station (Figure 16). A possible exception occurred at PC-3 where the peak number of species may not have been found in the analyses performed.

A study of the community composition on natural substrates in Prairie Creek showed shifts occurring between stations in the proportions of major periphyton groups present (Table 40). A large increase in the proportion of diatoms occurred at PC-3 with a compensatory drop in the proportion of green algae. A slight recovery toward upstream composition of greens was indicated at PC-4. Blue-greens and the red algae, Batrachospermum (Other) comprised a larger portion of the community at PC-1 than at any other station. Recovery was not indicated downstream.

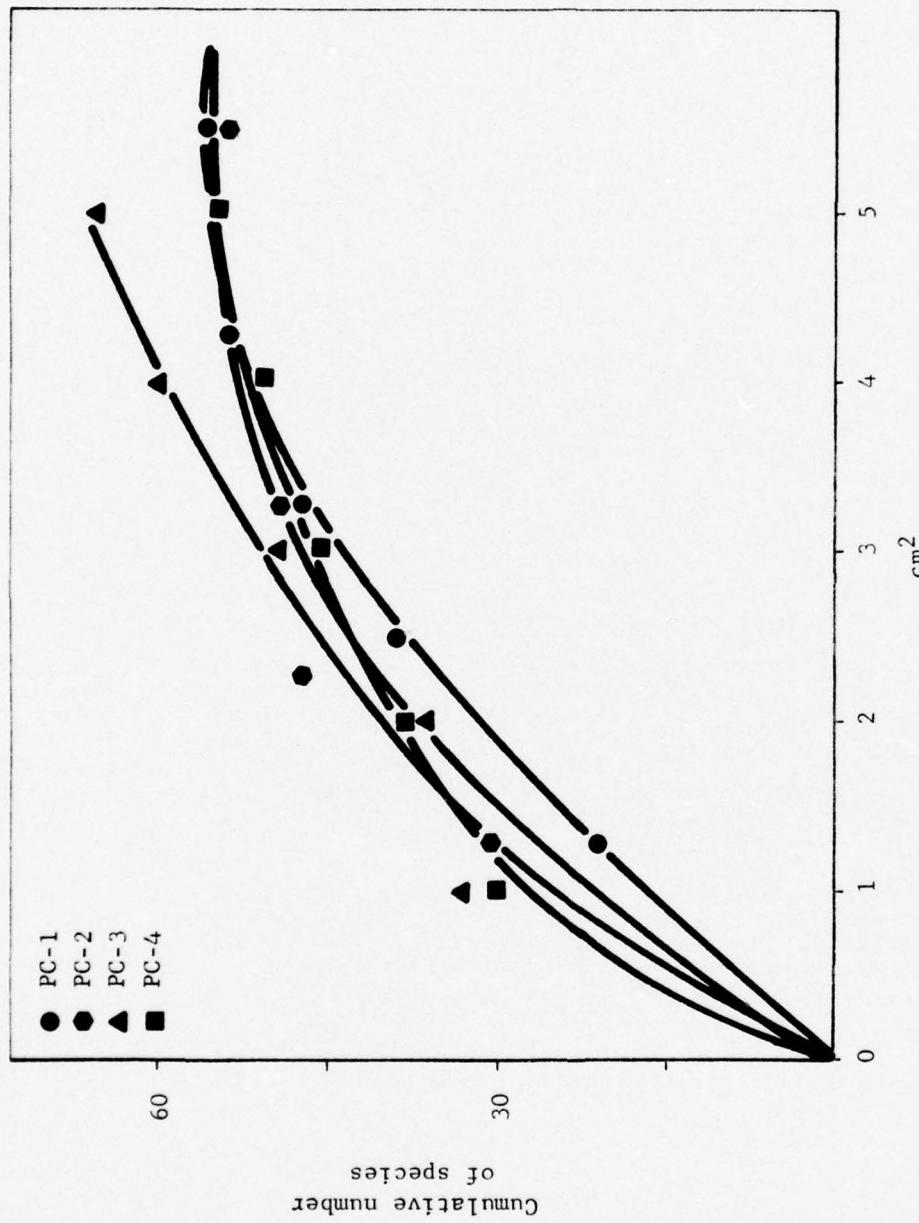


FIGURE 16. SPECIES-AREA CURVES FROM PRAIRIE CREEK PERIPHYTON DATA (NATURAL SUBSTRATES),  
FALL, 1975

TABLE 40. PROPORTIONS OF PERIPHYTON GROUPS ON NATURAL SUBSTRATES IN PRAIRIE CREEK, FALL, 1975

|      | Chrysophyta<br>(Diatoms) | Chlorophyta<br>(Greens) | Cyanophyta<br>(Blue-greens) | Flagellates | Other |
|------|--------------------------|-------------------------|-----------------------------|-------------|-------|
| PC-1 | .62                      | .14                     | .11                         | .03         | .10   |
| PC-2 | .80                      | .10                     | .05                         | .02         | .02   |
| PC-3 | .94                      | .02                     | .03                         | .007        | .007  |
| PC-4 | .89                      | .06                     | .04                         | .01         | .002  |

Biomass, Chlorophyll a, and Autotrophic Index. Ash-free biomass and chlorophyll a determinations and the calculated autotrophic index from Prairie Creek samples are presented in Table 41.

TABLE 41. BIOMASS, CHLOROPHYLL a, AND AUTOTROPHIC INDICES FROM PRAIRIE CREEK, FALL, 1975

|      | Biomass<br>mg/m <sup>2</sup> | Chlorophyll <u>a</u><br>mg/m <sup>2</sup> | Autotrophic Index<br>Biomass/Chl <u>a</u> |
|------|------------------------------|---|---|
| PC-1 | 822.4                        | 1.20                                      | 685.9                                     |
| PC-2 | 930.0                        | 2.59                                      | 358.7                                     |
| PC-3 | 2798.6                       | 15.73                                     | 178.0                                     |
| PC-4 | 1993.5                       | 10.91                                     | 182.8                                     |

Biomass increased by a factor of 3 at PC-3 with corresponding increases in chlorophyll a. Resultant indices approach an acceptable level of 100 at both PC-3 and PC-4. Biomass at PC-1 and PC-2 was lower than downstream values as was indicated by the lower numbers of individuals and species found there.

(Appendix C, Tables 46 through 50). However, the levels of chlorophyll a were proportionately much lower at the control stations than at the study stations. Table 40 shows that the proportions of unicellular flagellates was slightly higher at PC-1 and PC-2 than at downstream stations. These organisms may be heterotrophic--not contributing to the chlorophyll content yet adding biomass--but do not appear to have been present in large enough numbers to have been the sole cause for the low chlorophyll a values. Dinophyceans (Pyrrhophyta) also occurred at PC-1. These organisms contain predominantly brown pigments and, due to the wall structure of Peridinium in particular, can contribute much biomass. Batrachospermum (Rhodophyta) was found at both PC-1 and PC-2. This alga is a bulky specimen which may have added considerable biomass with correspondingly little chlorophyll a. However, there appears to be little in the data which supports the cause of the low chlorophyll a values and high indices.

Colonization. Numbers of individuals and species colonizing the artificial substrates in Prairie Creek showed fairly steady increases at PC-1, PC-2, and PC-4 during the three week incubation period indicating that complete colonization had not occurred at these stations. The increases were not proportionately consistent between stations. PC-3 had reached its peak number of individuals during the first week and maintained populations larger than the entire incubation period. This station had large populations of Melosira varians, Navicula cryptocephala, N. salinarum, Nitzschia amphibia which were maintained during the interval. A decrease in N. amphibia was primarily responsible for the decrease in total numbers of individuals at PC-3. As was seen at the other Prairie Creek stations, the greatest number of species occurred at PC-3 during the third week (Table 42).

TABLE 42. MEANS OF THE PERIPHYTON DATA FROM ARTIFICIAL SUBSTRATES  
IN PRAIRIE CREEK, FALL, 1975

|      | Week 1                                   |                        |                        | Week 2                                   |                        |                        | Week 3                                   |                        |                        |
|------|--|------------------------|------------------------|--|------------------------|------------------------|--|------------------------|------------------------|
|      | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity | Mean Number of Individuals $\times 10^4$ | Mean Number of Species | Mean Species Diversity |
| PC-1 | 0.9                                      | 18.0                   | 2.46                   | 2.6                                      | 25.0                   | 2.77                   | 8.4                                      | 25.0                   | 1.37                   |
| PC-2 | 2.4                                      | 13.0                   | 2.23                   | 2.5                                      | 23.3                   | 2.53                   | 4.8                                      | 29.0                   | 2.86                   |
| PC-3 | 15.3                                     | 27.0                   | 2.71                   | 12.9                                     | 21.3                   | 2.33                   | 12.4                                     | 38.0                   | 2.67                   |
| PC-4 | 5.5                                      | 27.0                   | 2.50                   | 5.1                                      | 26.0                   | 2.56                   | 7.5                                      | 40.0                   | 2.73                   |

Dendograms of the cluster analyses preformed on periphyton data from natural and artificial substrates in Prairie Creek appear in Figure 17, A and B, respectively.

The dendrogram for natural substrate data was formed from the clustering of stations at the following levels of similarity: PC-3 and PC-4 at .40, PC-1 and PC-2 at .32, these groups merging at .23. This clustering demonstrates a difference in the species assemblages upstream of the JAAP outfall from those downstream.

The dendrogram groupings for the data from artificial substrate do not reflect any expected pattern. This implies that periphyton communities on artificial substrates showed no adverse impact from the effluent to Prairie Creek.

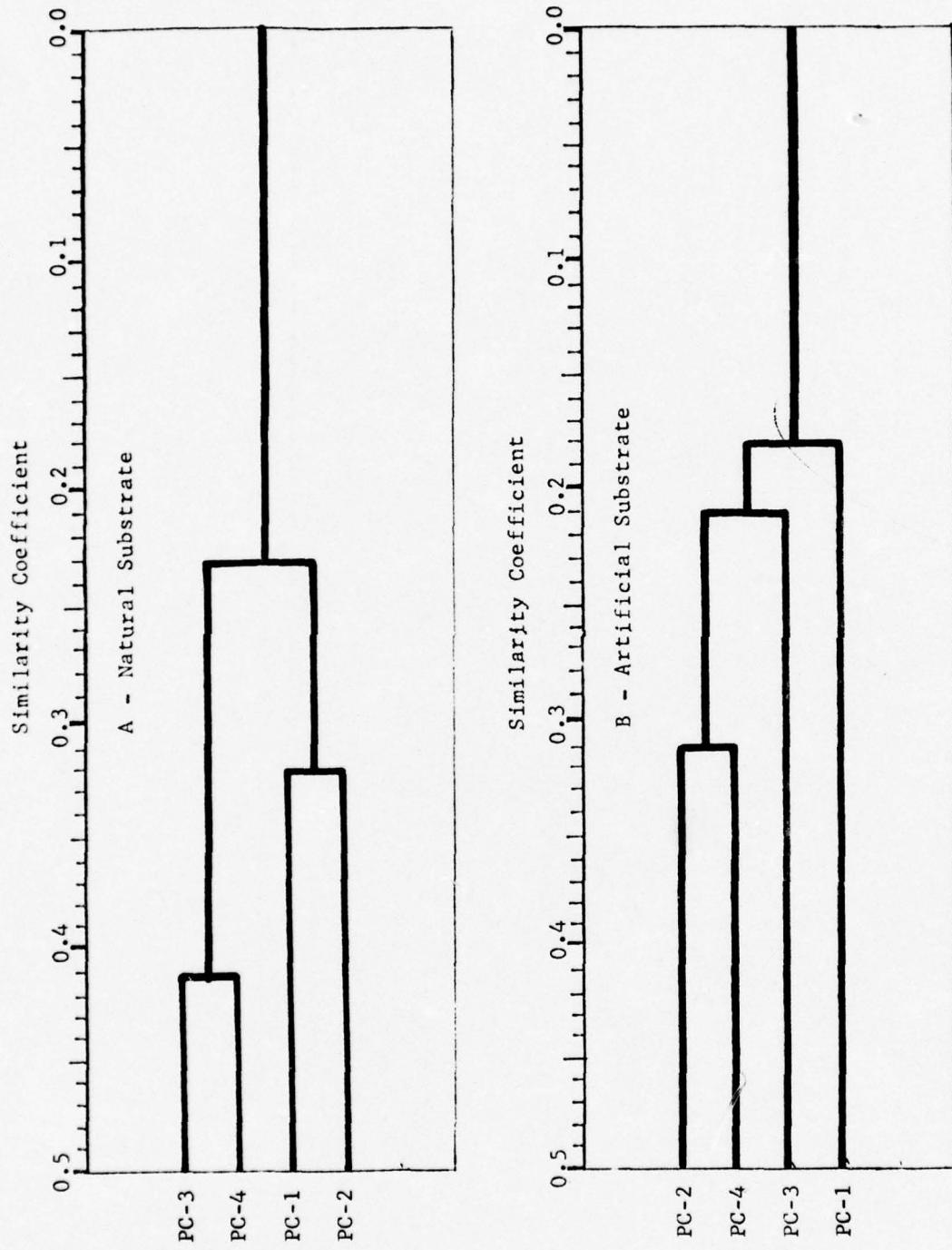


FIGURE 17. DENDOGRAM OF PRAIRIE CREEK PERiphyton DATA COLLECTED FROM NATURAL (A) AND ARTIFICIAL (B) SUBSTRATES, FALL, 1975

Prairie Creek Benthic Macroinvertebrates (Fall)

Species Composition. The benthic macroinvertebrate communities upstream and downstream of the Group 1 LAP line effluent outfall are composed of numerous species of several orders. The greatest number of species were Dipterans (mainly Chironomids), Ephemeropteron naiads and Trichopteran larvae. A total of 34 species were collected in Surber samples upstream at stations PC-1 and PC-2. Samples taken immediately downstream from the outfall (PC-3 and PC-4) contained 30 different species of invertebrates. The sampling site furthest downstream from the LAP outfall (PC-5) had 27 species of invertebrates. The number of invertebrate species collected with artificial substrate samplers from upstream (PC-1 and PC-2) and downstream (PC-3 and PC-4) were similar, being 27 and 33, respectively. Complete data sets are presented in Appendix D, Tables 41 through 50 for natural and artificial substrate samples.

The most dominant organisms inhabiting the natural substrates in the area of the LAP outfall were two Trichoptera larvae, Hydropsyche sp. and Cheumatopsyche sp.. Interestingly, Cheumatopsyche sp. was more abundant upstream from the LAP outfall while downstream Hydropsyche sp. was collected in greater numbers. This shift in dominance from Cheumatopsyche sp. to Hydropsyche sp. downstream of the LAP effluent discharge was also reported by Cooper, et al. (1975) in a previous study.

Another alteration in species composition is the noticeable reduction in the number of fingernail clams, Sphaerium sp., found in the substrates below the LAP discharge. At the upstream stations PC-1 and PC-2, 19 and 65 specimens of this clam were collected in Surber samples. At site PC-3 no clams were collected and only 8 individuals were found at station PC-4. Station PC-5 collections contained 16 specimens, indicating a recovery of this species downstream.

A review of the benthic macroinvertebrate species composition indicates some minor population shifts below the addition of the LAP effluent to Prairie Creek. These shifts may be due to slight changes in available habitats and/or the addition of LAP wastes.

Community Structure. Community structure parameters including number of individuals and species per sample area and species diversity were calculated from invertebrate collection data from Prairie Creek (Table 43). Samples from station PC-5 were not averaged with other downstream samples because of the distance downstream from the outfall. This site was intended as an indicator of recovery in the event large differences occurred between the upstream and downstream stations in the immediate area of the outfall. Due to the similarity of the collections in the outfall area PC-5 collections have been excluded from some of the calculations and comparisons in this section. A review of Table indicates little change in the invertebrate community downstream from the LAP effluent discharge. The greatest difference is observed in the natural substrates at station PC-5 where numbers of species and individuals per sample area were considerably higher. This situation was most likely due to morphological stream changes creating a greater number of habitat niches. Collections from artificial substrate samples show the invertebrate communities at all sites to be composed of similar numbers of individuals and species.

Table 44 presents the results of ANOVA performed on benthic macro-invertebrate data to test for differences between stations located upstream and downstream of the LAP outfall to Prairie Creek. Samples from PC-5 were not used in the ANOVA.

The natural substrate data showed no significant differences in any of the variables analyzed. Similarly, only three of the ANOVA performed on the artificial substrate data were significant. Among these three, two of them (numbers of species during week 2, and diversity indices during week 3) appeared implausible because the absolute differences between the upstream and downstream means were so small.

The mean number of species at PC-3 and PC-4 was higher than that PC-1 and PC-2 for each of the three sampling weeks of artificial substrate data. Aside from this, there were no other consistent trends affecting the size relationships of the upstream and downstream means.

Figure 18 presents dendograms of cluster analyses performed on macroinvertebrate data from all five Prairie Creek stations, separately for natural (A) and artificial (B) samples. Both dendograms were formed by the same

TABLE 43. SUMMARY OF ANALYSES OF THE BENTHIC-MACRO INVERTEBRATE DATA FROM NATURAL AND ARTIFICIAL SUBSTRATE SAMPLES FROM PRAIRIE CREEK, FALL, 1975.

TABLE 44. ANOVA OF BENTHIC MACROINVERTEBRATE DATA  
FROM PRAIRIE CREEK, FALL, 1975

| Site                         | Natural Substrates    |                  |                  |                       |                  |              | Artificial Substrates |                  |                  |                       |                  |                  |
|------------------------------|-----------------------|------------------|------------------|-----------------------|------------------|--------------|-----------------------|------------------|------------------|-----------------------|------------------|------------------|
|                              | Week 1                |                  |                  | Week 2                |                  |              | Week 3                |                  |                  | Week 4                |                  |                  |
|                              | Number of Individuals | Species          | Site             | Number of Individuals | Species          | Site         | Number of Individuals | Species          | Site             | Number of Individuals | Species          | Site             |
| Fall--Prairie Creek          | PC-1-2<br>PC-3-4      | 88.0<br>103.1    | PC-1-2<br>PC-3-4 | 11.2<br>11.9          | PC-1-2<br>PC-3-4 | 1.59<br>1.80 | PC-3-4<br>PC-1-2      | 41.0<br>70.0     | PC-1-2<br>PC-3-4 | 8.0<br>9.7            | PC-1-2<br>PC-3-4 | 1.58<br>1.79     |
| Significance level of F-test | —                     | —                | —                | —                     | —                | —            | —                     | —                | —                | .95                   | —                | —                |
| <hr/>                        |                       |                  |                  |                       |                  |              |                       |                  |                  |                       |                  |                  |
| Site                         | Artificial Substrates |                  |                  |                       |                  |              | Artificial Substrates |                  |                  |                       |                  |                  |
|                              | Week 1                |                  |                  | Week 2                |                  |              | Week 3                |                  |                  | Week 4                |                  |                  |
|                              | Number of Individuals | Species          | Site             | Number of Individuals | Species          | Site         | Number of Individuals | Species          | Site             | Number of Individuals | Species          | Site             |
| PC-3-4<br>PC-1-2             | 35.8<br>40.3          | PC-1-2<br>PC-3-4 | 9.8<br>11.6      | PC-1-2<br>PC-3-4      | 1.90<br>2.12     | PC-3-4       | 27.0<br>46.7          | PC-1-2<br>PC-3-4 | 9.5<br>12.0      | PC-1-2<br>PC-3-4      | 1.85<br>1.87     | PC-1-2<br>PC-3-4 |
| Significance level of F-test | —                     | —                | —                | —                     | .99              | —            | —                     | —                | —                | —                     | —                | —                |

(1) No Surber taken at PC-1 because there were no riffles.

(2) No H-D's at PC-2 for this week.

— No significant difference.

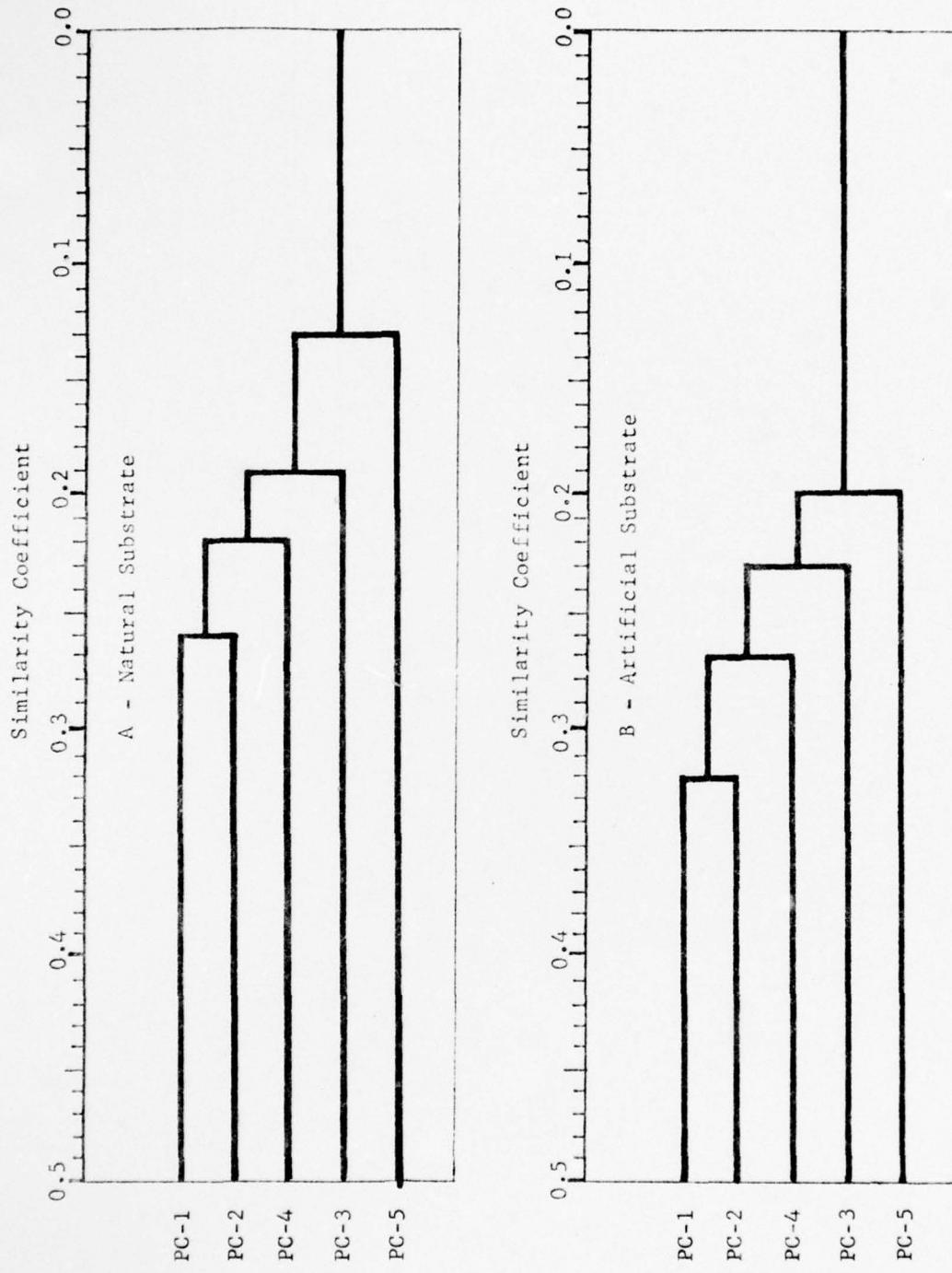


FIGURE 18. DENDOGRAM OF PRAIRIE CREEK BENTHIC MACROINVERTEBRATE DATA COLLECTED FROM NATURAL (A) AND ARTIFICIAL (B) SUBSTRATES, FALL, 1975

sequence of steps, which led to the following conclusions: (1) PC-1 and PC-2 have the most similar species assemblages out of all possible pairs of sites. (2) The downstream station PC-4 is more similar to PC-1 and PC-2 than it is to PC-3. (3) PC-5 is different from all other Prairie Creek stations.

Conclusion (3) above is to be expected, because PC-5 is a long distance from stations 1-4. Also, conclusion (2) may indicate that the species assemblages at PC-3 are slightly affected by the LAP outfall, and that PC-4 is an area of recovery.

The colonization rates (Table 44) of artificial substrate samples placed in Prairie Creek were not presented graphically because peak numbers of both individuals and species were reached before the first set of samples were retrieved. There was actually a decline in number of individuals with number of species remaining constant or increasing slightly over the 3 week incubation period. This reduction appears to be due to the emergence of some individuals of several midge species before the final samples were collected.

ASSOCIATION OF MUNITIONS EFFLUENTS WITH ECOLOGICAL RESPONSE

The discharge of TNT manufacturing wastes to the environment indirectly adds a variety of other degradation products to receiving systems. Two of these compounds, 2,4-DNT and 2,6-DNT, were quantified in water and sediment samples from JAAP. Concentrations of "pink water" used in the following discussion will refer to the summed concentrations of 2,4-DNT, 2,6-DNT and TNT.

Product-moment correlation coefficients (Pearson's  $r$ ) were computed between pink water constituent levels in the water and each water quality parameter. Values from all available water samples taken at all stations were used except for those taken at the outfalls; these were excluded from the calculations since the combinations of values there did not represent stable conditions.

Pink water constituent levels were found to have significant positive correlations with the following water quality parameters: hardness ( $r = .61$ ), dissolved solids ( $r = .50$ ),  $\text{NO}_2$  ( $r = .66$ ),  $\text{SO}_4$  ( $r = .54$ ), and conductivity ( $r = .44$ ). These  $r$  values were each calculated from 22 points and were all significant at the .95 level or greater. No significant negative correlations were found.

Algae

Periphyton communities at the Grant Creek and Doyle Lake stream stations indicate adverse or perturbed environmental conditions during the spring and fall surveys. Isolating the periphytic response due solely to munitions process wastes in these systems was complicated by low flows and by confounding effects from the filtration plant effluent during the fall. The low munitions concentrations in the LAP effluent to Prairie Creek in the fall had little effect and were a possible source of enrichment for the periphyton.

Comparisons between each of the three community parameters investigated and concentrations of pink water constituents (PWC) were studied. Numbers of species were found to vary slightly at differing PWC concentrations. Numbers of individuals of certain species were some-

times found to increase with PWC levels. An inverse relationship between diversity and PWC concentration was indicated in those data relatively uncomplicated by external factors. Therefore, species diversity was chosen as the parameter best suited for determining community response.

All figures were constructed by plotting the mean species diversities of the periphyton communities versus the PWC concentration at each stream station. Doyle Lake stations representing pond habitats (DL-2 and DL-3) were excluded from these figures. Reference stations appear on the left side of each figure at zero (0) concentrations.

Figure 19 presents the data from artificial substrates from the fall survey. Diversities were not found to be affected by PWC at any of the stations sampled. The lowest diversities occurred at two stations, GC-3 and DL-1, where the filtration plant effluent effects and low flow, respectively, caused environmental stresses. These external factors did not appear to be expressed as strongly on the natural substrates (Figure 20). Mean diversities varied inversely with munitions concentration at Doyle Lake stream station, although there was considerable overlaps in the ranges. Natural substrates at DL-1 and DL-4 were selected from areas of adequate flow at the time of sampling. However, those substrates may have been exposed during prior lower flows and may not have been fully colonized. The filtration plant effluent did not appear to greatly impact the natural substrate at GC-3, as all control stations had similar diversities. The study stations in Grant Creek had similar diversities also. Diversity was found to be decreased at PWC concentrations of 40-65 ppb, although ranges overlapped somewhat with control stations also. The low concentrations in Prairie Creek resulted in no alteration of diversity on either substrate.

The high water levels of the spring survey lent some uniformity to stream habitats. In Grant Creek particularly, effects from the flocculant suspension in the filtration plant effluent were diminished. On the natural substrates, no adverse effect appeared in species diversities at concentrations as high as 116 ppb in Grant Creek (Figure 21). Mean diversity was considerably lower at DL-1 at 29 ppb than at DL-4 at 6 ppb. Differences here were likely due to morphological variation between stations as this response was not found at higher concentrations.

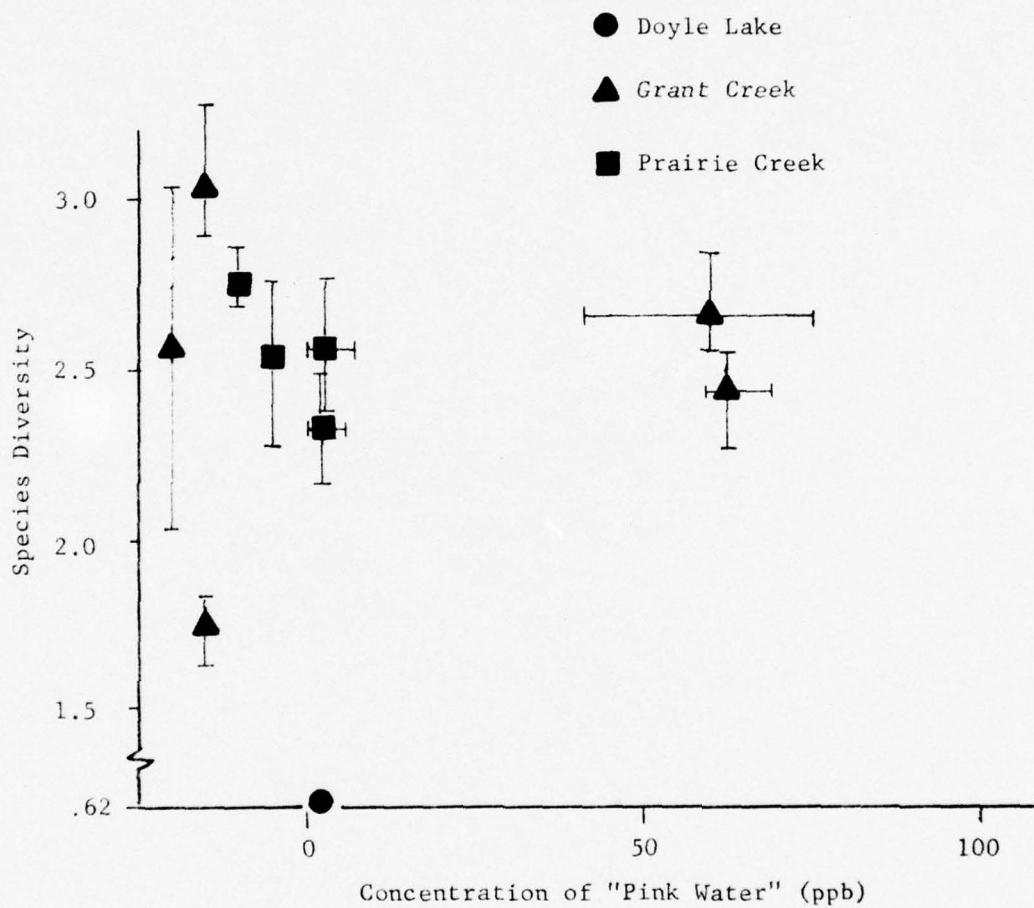


FIGURE 19. ALGAL SPECIES DIVERSITY (ARTIFICIAL SUBSTRATES) VERSUS "PINK WATER" CONCENTRATIONS IN WATER AT JAAP, FALL, 1975

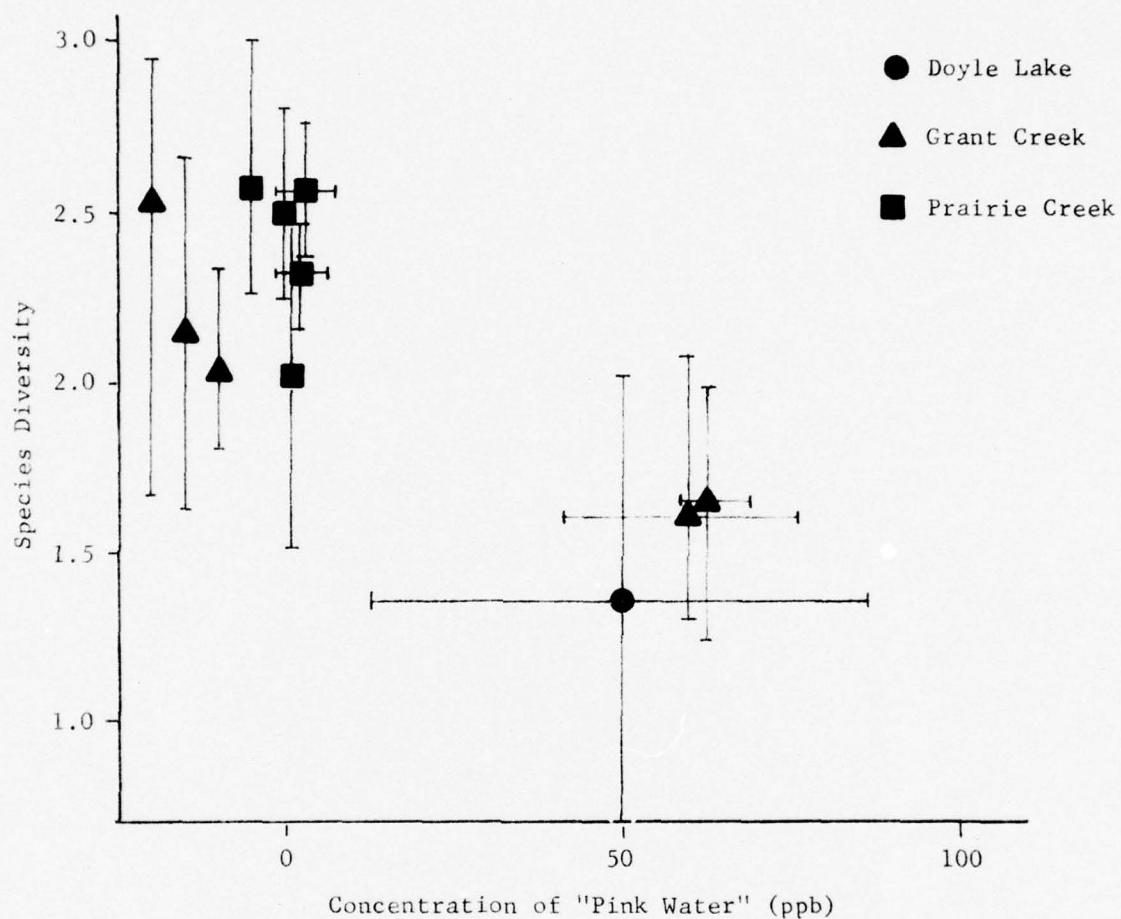


FIGURE 20. ALGAL SPECIES DIVERSITY (NATURAL SUBSTRATES) VERSUS "PINK WATER" CONCENTRATIONS IN WATER AT JAAP, FALL, 1975

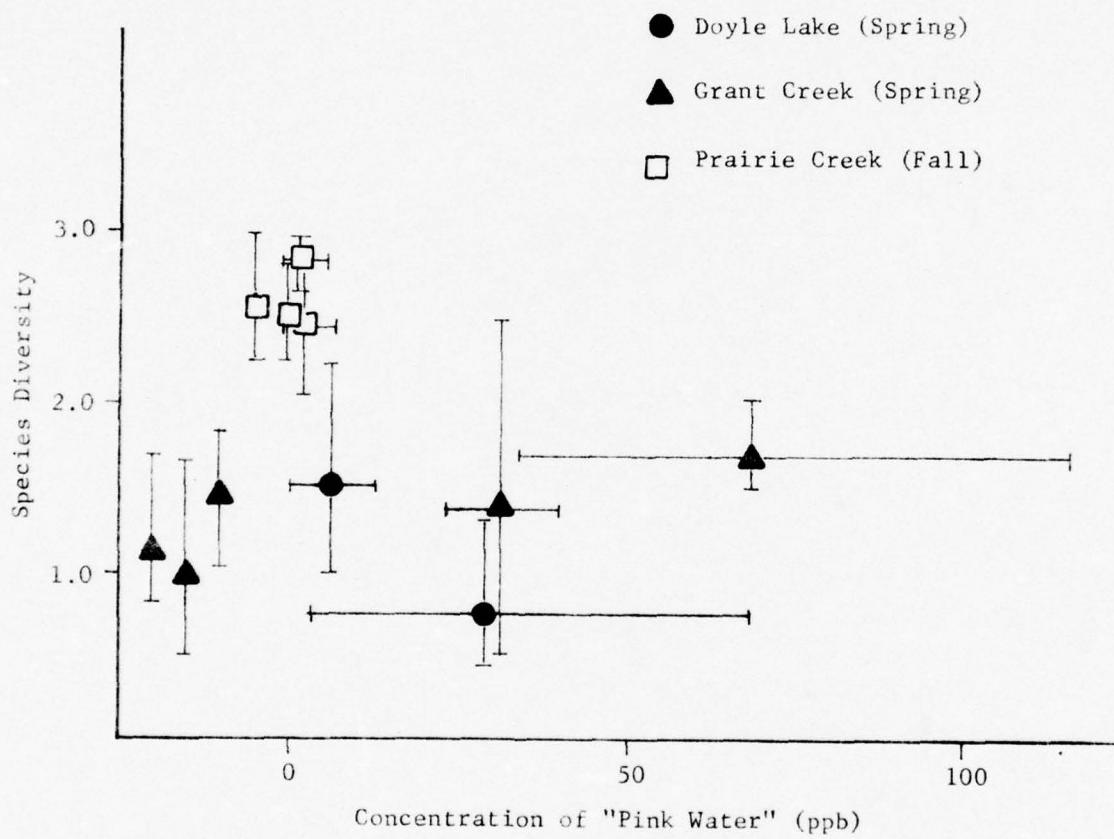


FIGURE 21. ALGAL SPECIES DIVERSITY (NATURAL SUBSTRATES) VERSUS "PINK WATER" CONCENTRATIONS IN WATER AT JAAP

On artificial substrates (Figure 22), diversity was again greatly depressed at DL-1 (4-68 ppb) with no overlap in the ranges of the two Doyle Lake stations. A slight depression was also indicated in Grant Creek diversities in the same range of PWC concentrations (22-40 ppb). (The point for GC-4 resulted from analyses of one sample since that sampler was lost; hence, no range appears about this value.)

Shifts in species composition are not evident in species diversity indices. Such population shifts occurred in study station communities. Tolerant species, normally present even at control stations increased dramatically in numbers with the addition of munitions compounds. Three species of extreme tolerance - Navicula cryptocephala, Nitzschia amphibia, and Nitzschia palea (Palmer, 1969) - were investigated for their individual responses to increased PWC concentrations. Bar graphs of the average number of individuals  $\text{cm}^{-2}$  for each of the three species were constructed. Graphs presented were constructed from data from natural substrates. Similar patterns were observed from artificial substrate data.

Figure 23 is a bar graph of the data from the spring survey. Doyle Lake stream stations were included with Grant Creek as flows were high during this period lending some uniformity to habitat types. A distinct correlation between species population shifts and PWC concentration is illustrated in this graph. A minor response is indicated at very low concentrations (mean 2.4 ppb at DL-4) becoming more pronounced with higher PWC levels. Uniformity among control stations also appears good.

Data from the fall survey appears in Figure 24. Prairie Creek is included in this graph because it received the LAP effluent during the fall survey. The correlation between population shifts and PWC concentration is again distinct even at low concentrations. One control station, GC-1 appears atypical, but the total population of the three tolerant species remains lower than the total at GC-5. The dramatic increases between PC-2 and PC-3 may be due partially to the munitions compounds entering in the LAP effluent. However, the LAP effluent also contains detergent wash water with elevated phosphate levels. The increased populations may be associated with the additional availability of this nutrient.

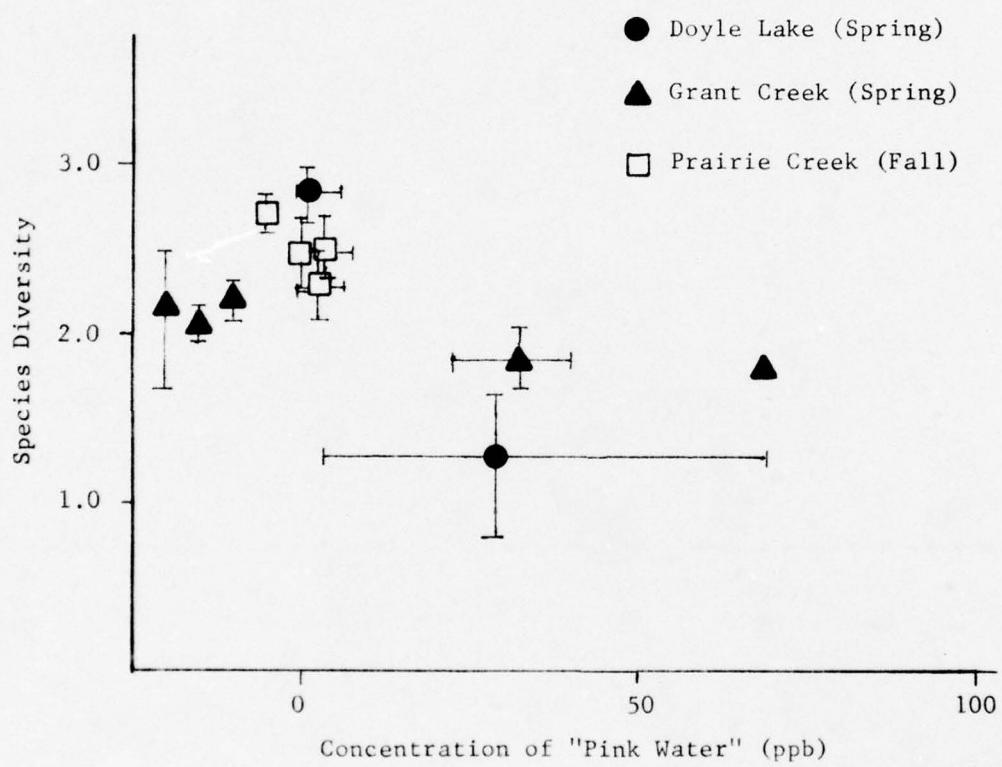


FIGURE 22. ALGAL SPECIES DIVERSITY (ARTIFICIAL SUBSTRATES) VERSUS  
"PINK WATER" CONCENTRATIONS IN WATER AT JAAP

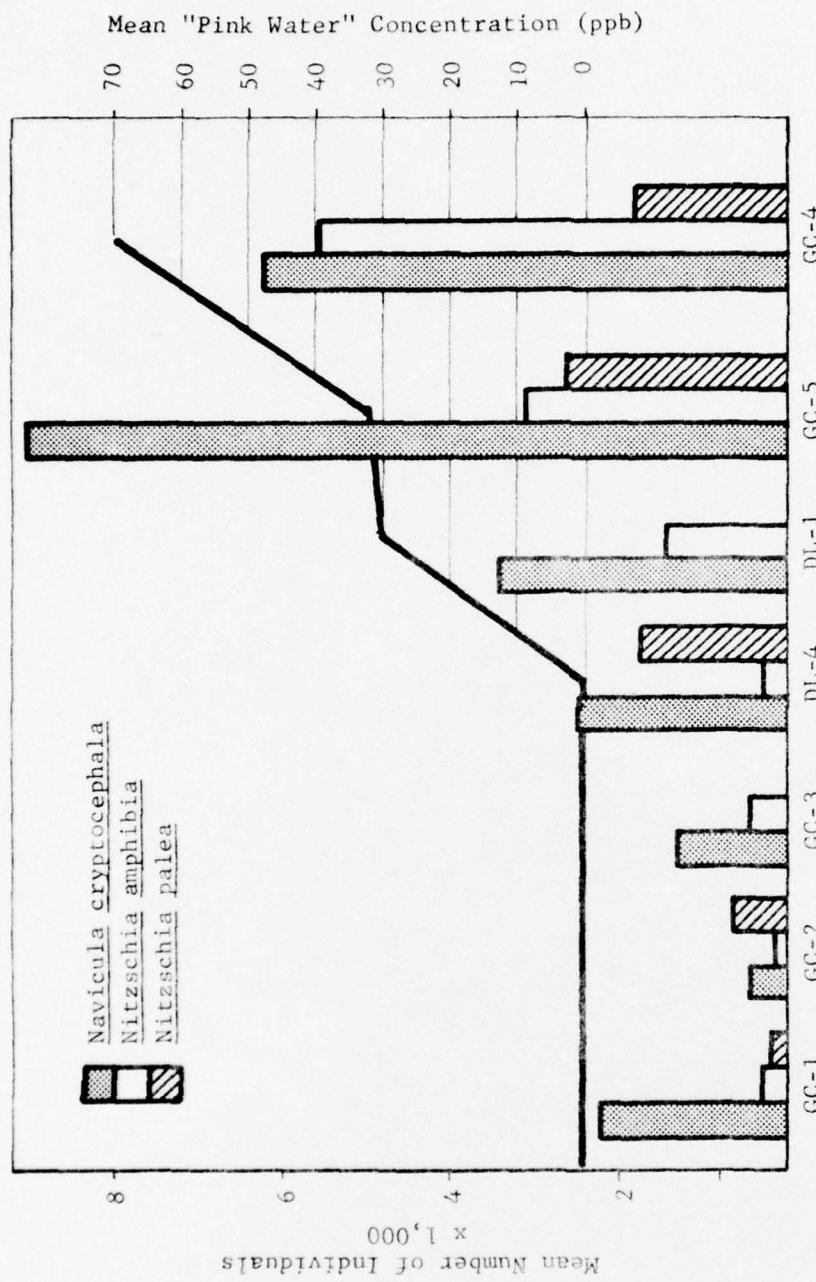


FIGURE 23. NUMBERS OF INDIVIDUALS OF THREE TOLERANT PERIPHYTON SPECIES (NATURAL SUBSTRATES) AS EFFECTED BY "PINK WATER" CONCENTRATION, SPRING, 1975

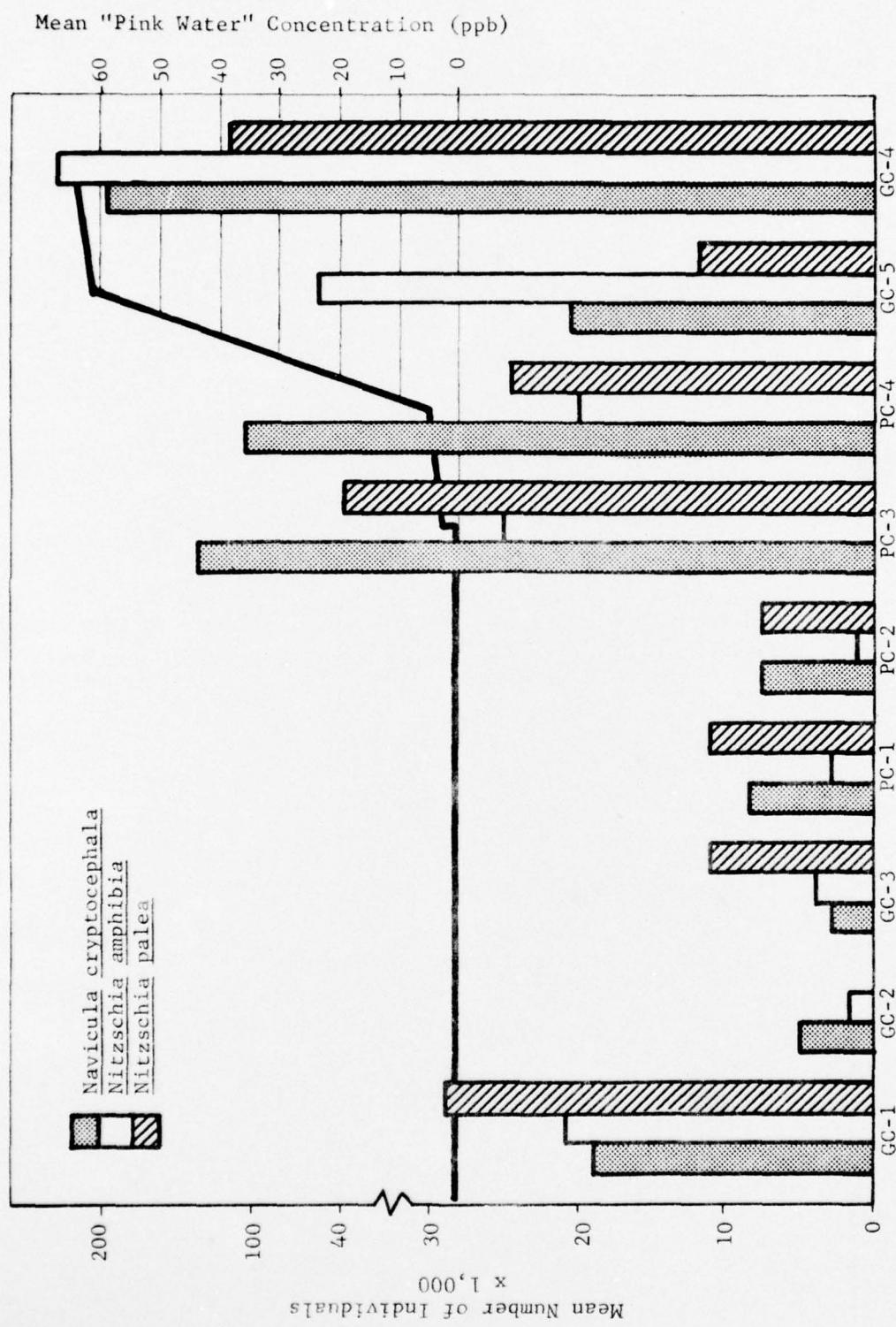


FIGURE 24. NUMBERS OF INDIVIDUALS OF THREE TOLERANT PERIPHYTON SPECIES (NATURAL SUBSTRATES) AS EFFECTED BY "PINK WATER" CONCENTRATION, FALL, 1975

No relationship between other water quality parameters and species population increases could be drawn. Nutrients, primarily nitrogen compounds and phosphates, were in similar concentrations at both control and study stations (with the exception of the previously discussed phosphate increase in Prairie Creek).

The observed community response may be due to inhibitory effects of PWC on sensitive species allowing tolerant species to be more competitive and flourish. Also, these tolerant species may more readily use the increased nitrogen-containing compounds available in the form of TNT, 2,4 DNT 2,6 DNT or other degradation products. The correlation between PWC and population shifts may be due to either of these reasons, or others not suggested by the data.

#### Benthic Macroinvertebrates

Benthic macroinvertebrate communities inhabiting aquatic environments in the area of the Joliet Army Ammunition Plant are adversely impacted by waste discharges from the manufacture of TNT and the loading and packing of compound B. Invertebrate communities found in Grant Creek below the water treatment plant (GC-3) were depauperate and unstable. This environmental stress produced by the suspended flocculant material contained in the treatment plant effluent extended as far downstream as station GC-5 during periods of low flow. Many of the effects observed at the stations below the "pink water" addition were confused or overshadowed by the physical stress of this suspended material.

The situation in Doyle Lake also creates tenuous results. The two stream stations studied in this area were quite dissimilar and DL-1 was often without flow, dependent upon plant operations. This intermittent condition could be responsible for the depauperate invertebrate community found there.

Prairie Creek data provides excellent information about the effects of low concentrations of pink water on benthic macroinvertebrates. These effects were usually subtle and/or limited to a single species of aquatic invertebrate.

Another complicating factor arises from the fact that sediment concentrations of PWC were between 10-40 times higher than those found in the water. It appears that these compounds can and do accumulate in the bottom sediments of these aquatic environments.

With these limitations taken into consideration, a discussion of biological effects observed at various PWC concentrations follows:

Data from natural substrate collections were used to calculate station means for the number of individuals, number of species and species diversity indices which were compared to station mean PWC concentrations. Figure 25 presents the plot for species diversity ( $\bar{H}$ ) versus PWC concentration. A review of points from the same streams demonstrates little or no reduction in diversity in Prairie Creek and Grant Creek with increased PWC concentrations. A reduction in diversity is evident in the Doyle Lake stream stations at PWC concentrations in the range of 50.0 ppb. Similar patterns are evident for the number of species when plotted against PWC concentrations (Figure 26). The mean numbers of individuals per sample are compared with PWC concentrations in Figure 27. Here again little effect was seen in Prairie Creek where concentrations of munitions wastes were less than 10 ppb. In addition to the reduction in numbers observed in Doyle Lake, a slight reduction in numbers also occurs at Grant Creek where PWC concentrations ranged from 25-116 ppb. These three plots indicate (with the exception of the Doyle Lake data) that little or no effect was exerted on the invertebrate community at PWC concentrations up to 116 ppb. Little emphasis was placed on the low values recorded from DL-1 samples in the spring in this interpretation because of the morphological differences between stations DL-1 and DL-4. DL-1 was essentially an effluent ditch subjected to continuous 100% LAP wastewater only.

The station means calculated from artificial substrate sample data confirm the results observed from the natural substrate plots with one exception. No reduction in number of individuals was observed below the industrial outfall to Grant Creek in the spring. As previously discussed, results from Grant Creek sampling during the fall were complicated by the water treatment plant effluent rendering those points on the previous curves invalid. No discussion of these points compared to PWC are warranted.

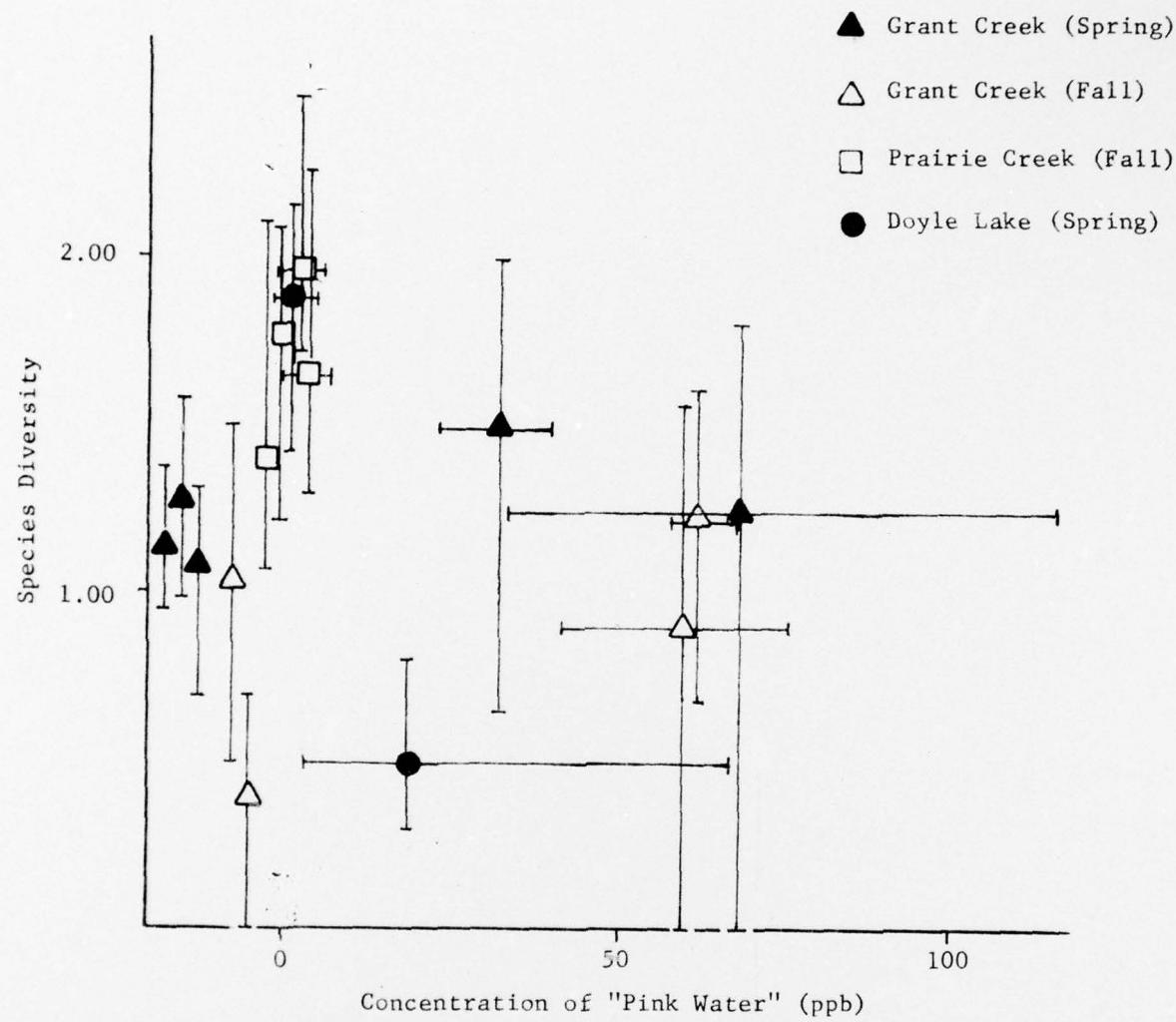


FIGURE 25. BENTHIC MACROINVERTEBRATE SPECIES DIVERSITY (NATURAL SUBSTRATES) VERSUS "PINK WATER" CONCENTRATIONS IN WATER AT JAAP

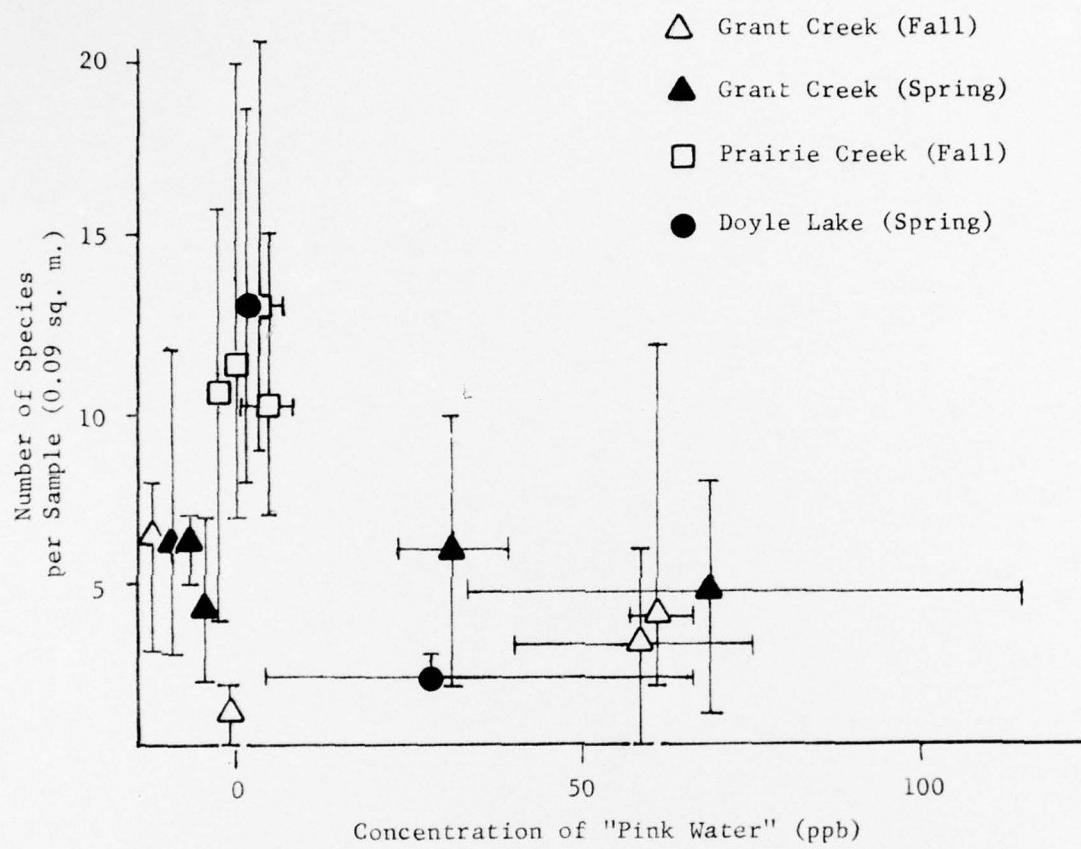


FIGURE 26. NUMBER OF BENTHIC MACROINVERTEBRATE SPECIES (NATURAL SUBSTRATES) VERSUS "PINK WATER" CONCENTRATIONS IN WATER AT JAAP

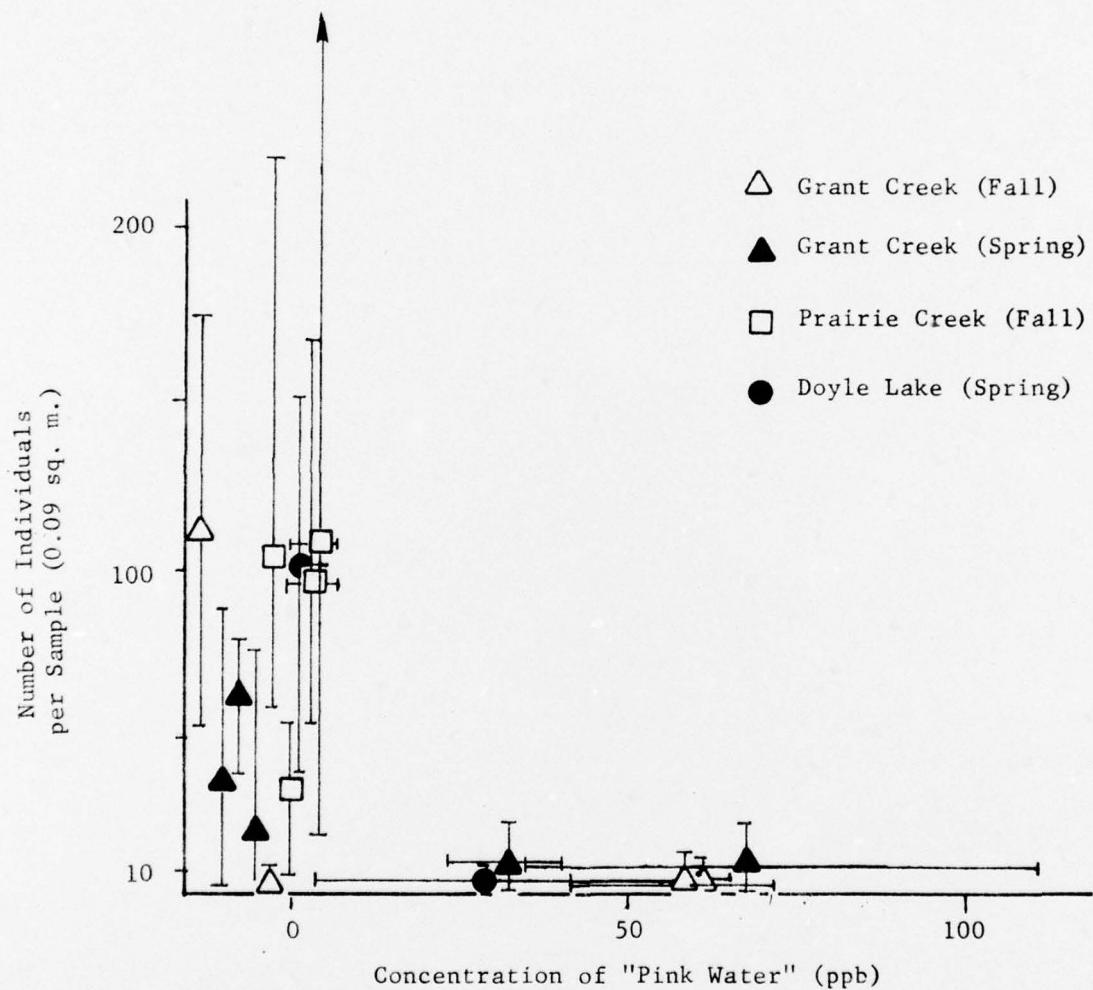


FIGURE 27. NUMBER OF BENTHIC MACROINVERTEBRATE INDIVIDUALS (NATURAL SUBSTRATES) VERSUS "PINK WATER" CONCENTRATIONS IN WATER AT JAAP

However, two specific cases of observed population changes can be cited from the collections from Grant Creek and another from Prairie Creek. In Grant Creek two dominant organisms (Asellus sp. and Simulium sp.) upstream from the pink water discharge were either totally eliminated in the case of Simulium sp. or found in much fewer numbers as with Asellus sp.. The reduction in numbers of Asellus sp. may be due to the increased water currents found in the channelized portion of Grant Creek below the industrial outfall. This species prefers slow moving water found in the pooled areas of natural streams. The elimination of Simulium sp. cannot be explained as easily; this species tends to prefer fast moving water. Its elimination was most likely due to the "pink water" being discharged to Grant Creek.

The population size reduction of the fingernail clam, Sphaerium sp. in Prairie Creek may be caused by the addition of loading and packing wastes to this stream. Figure 28 presents the numbers of individuals collected in each Surber sample from Prairie Creek stations. As seen in the curve, no clams were collected below the discharge at station PC-3 where PWC concentrations in water and sediment had mean values of 3.2 ppb and 35.9, respectively. This reduction may or may not be due to PWC contained in the water.

Other population shifts such as the dominance shift of Cheumatopsyche sp. to Hydropsyche sp. below the LAP discharge to Prairie Creek have also been observed.

Another evidence of environmental perturbation in Doyle Lake was the total dominance of a single species of midge, Glyptotendipes sp..

The results from the two sampling missions to the Joliet plant have demonstrated adverse effects on the benthic macroinvertebrate communities receiving munitions manufacturing wastes. Many of these effects are subtle or limited to a single species of invertebrate. Concentrations in water during intensive sampling periods ranged from 0.0 to 116.9 ppb of PWC. It appears that effects on the invertebrate community begin to occur in this range of concentrations as samples analyzed from areas with these concentrations are measurably affected.

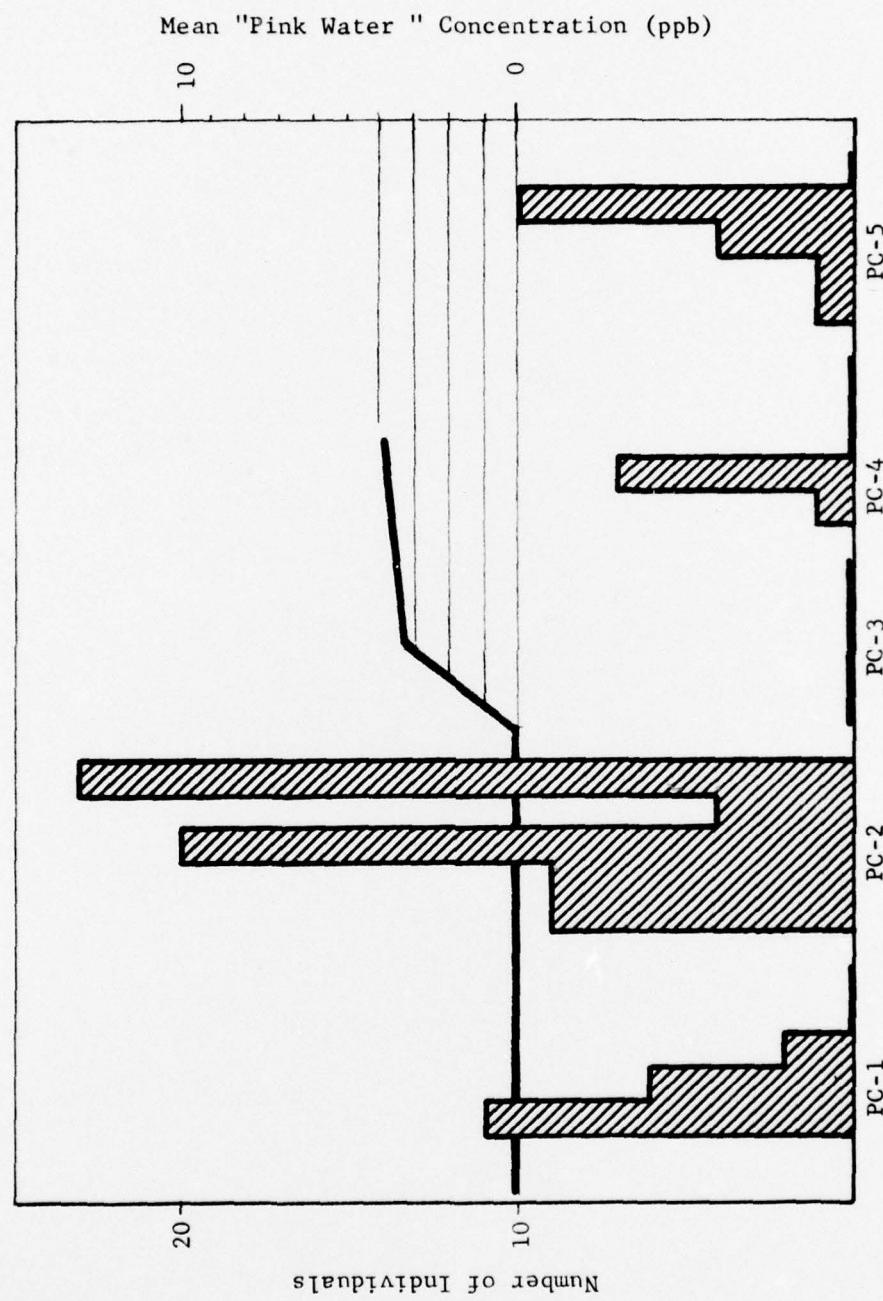


FIGURE 28. NUMBERS OF INDIVIDUALS OF SPHAERIUM SP. (NATURAL SUBSTRATES AS EFFECTED BY "PINK WATER" CONCENTRATIONS, FALL, 1975

CONCLUSIONS RELATIVE TO ENVIRONMENTAL QUALITYStandards

The previous section discussed the relationships between "pink water constituents" concentrations and various ecological parameters measured throughout Phase II. These data and relationships were derived exclusively from field measurements, where a multitude of physical, chemical, and ecological variables interact in an unknown manner. Therefore, no conclusions as to causality can be drawn from the results of this research effort.

Periphyton and benthic macroinvertebrates on natural and artificial substrates exposed to "PWC" concentrations ranging from zero to 10.0 ppb in Prairie Creek experienced little or no adverse ecological effects.

Concentrations of "PWC" in the range of 50-100 ppb in Grant Creek and Doyle Lake water were associated with environmental perturbances reflected in periphyton, phytoplankton, and benthic invertebrate community changes. Sediment concentrations in the areas of greatest observed effects ranged between 10 and 40 times higher than those found in the water.

In conclusion, "PWC" (TNT, 2,4 DNT and 2,6 DNT) concentrations in water in the range of 50-100 ppb are associated with ecological changes in the benthic macroinvertebrate and algae communities of the aquatic ecosystems investigated. Therefore, a "no effect" threshold would appear to be at some concentration below 50-100 ppb range.

Stream Versus Effluent Concentration Considerations

"No effect" levels of "PWC" probably lie in the range of 50-100 ppb for macroinvertebrates and algae. The transformation of "no effect" environmental concentrations to "no effect" effluent loading rates cannot be made without considering the effluent receiving system in question. In the case of TNT and its degradation products, their relatively high solubility in water (greater than the toxic levels indicated by this study) require specific knowledge as to dilution and dispersion in each receiving system before "no effect" effluent loading rates can be determined.

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AQUATIC LIFE FIELD STUDIES AT JOLIET ARMY AMMUNITION PLANT. VOL--ETC(U)  
AUG 76 J M STILWELL, D C COOPER, M A EISCHEN      DAMD17-74-C-4123  
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# **SUPPLEMENTARY**

# **INFORMATION**

**ERRATA**

**AD-A033 548**

The Appendices, Vol II contained raw data only and was never published.  
Any/all references to Appendices, Volume II that appear in this  
Volume I should be deleted.

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**5 May 80**